

Natural Product Inspired Synthesis of Cysteine-derived Tetramates for Antibacterial Activity



A thesis submitted in partial fulfilment of the requirement for the degree of
Doctor of Philosophy in Organic Chemistry

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Declaration

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Date: 3rd Jan 2021

Abstract

Natural product inspired synthesis of cysteine-derived tetramates for antibacterial activity

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This thesis describes the synthesis and evaluation of cysteine-derived tetramate (pyrrolidine-2,4-dione) derivatives for the development of novel antibacterial drugs.

Chapter 1 gives a brief overview of the history of antibiotics and clinically available antibacterial drugs. It addresses the need for novel antibacterial drugs due to the emergence of bacterial resistance. While exploring different chemical approaches to tackle the problem of drug resistance, it highlights the re-emergence of natural products as a crucial source for drug development. In particular, it focuses on tetramic acid containing natural products and discusses their value in antibacterial drug discovery, their properties and synthesis. In the end, it exposes the need for optimisation of cysteine-derived tetramates due to their apparent reduction of antibacterial activity when tested *in vitro* in the presence of blood, hence establishing the hypotheses and aims of this project.

Chapter 2 describes the synthesis, structural and brief antibacterial evaluation of a library of cysteine-derived tetramates with increased hydrophilicity by incorporation of pendant heterocyclic rings. An effective synthetic protocol for accessing polar tetramate derivatives is established and a computational study has developed detailed understanding of the controlling factors of the key Dieckmann cyclisation step. Direct evidence of strong metal chelation in these tetramate systems is also demonstrated.

Chapter 3 and 4 discuss chemical modifications on cysteine-derived tetramates to modulate their metal chelation property. Structural dependency of the metal binding ability is established, with a library of cysteine-derived tetramates exhibiting evidence for reduced metal chelation. The antibacterial activity of these tetramates is evaluated in relation with their metal binding ability.

Chapter 5 provides a detailed biological evaluation of the synthesised cysteine-derived tetramates with regard to their antibacterial potency, physicochemical properties and metal chelation ability. The potential for multi-targeting mechanisms coupled with a low cytotoxicity is also briefly addressed. It demonstrates the types of structural modifications possible to assess hydrophilic cysteine-derived tetramates with reduced metal chelation, while preserving their selective Gram-positive activity with MIC against MRSA < 8 µg/ml.

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Abbreviations

1D	1-dimensional
2D	2-dimensional
Å	angstrom
Ac	acetyl
AGP	α_1 -acid glycoprotein
AIBN	azobisisobutyronitrile
APCI	atmospheric-pressure chemical ionization
aq.	aqueous
Ar	aromatic
ATR	attenuated total reflection
Boc	<i>tert</i> -butyloxycarbonyl
<i>B. subtilis</i>	<i>Bacillus subtilis</i>
Bu	butyl
<i>c</i>	concentration
CaCo	human epithelial colorectal adenocarcinoma cells
°C	Celsius
CDI	1,1-carbonyldiimidazole
CDC	Centres for Disease Control and Prevention
CFU	colony-forming unit
clogD _{7.4}	calculated distribution coefficient between octanol and water at pH=7.4
clogP	calculated partition coefficient between octanol and water
CtD	complexity-to-diversity
d	day
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	<i>N,N'</i> -dicyclohexylcarbodiimide
DCM	dichloromethane
DEAD	diethyl azodicarboxylate
DFT	density functional theory
DIBAL-H	diisobutylaluminium hydride
DIPEA	<i>N,N</i> -diisopropylethylamine
DMAP	4-dimethylaminopyridine
DME	dimethoxyethane
DMF	<i>N,N</i> -dimethylformamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
dr	diastereomeric ratio
DTT	dithiothreitol
EA/EtOAc	ethyl acetate
<i>E. coli</i>	<i>Escherichia coli</i>

EDCI	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
ee	enantiomeric excess
ent	enantiomer
eqv.	equivalent
ESI	electrospray ionization
Et	ethyl
FBDD	fragment-based drug discovery
Fmoc	fluorenylmethoxycarbonyl
FPP	farnesyl pyrophosphate
FT	fourier transformation
h	hour
HeK-293	human embryonic kidney 293 cells
<i>H. influenza</i>	<i>Haemophilus influenza</i>
HMBC	¹ H- ¹³ C heteronuclear multiple bond correlation spectroscopy
HOBt	1-hydroxybenzotriazole
HPLC	high performance liquid chromatography
HRMS	high-resolution mass spectrometry
HSA	human serum albumin
HTS	high-throughput screening
Hz	Hertz
IC ₅₀	concentration required to achieve 50% inhibition in a cellular assay
ICP-MS	inductively coupled plasma mass spectrometry
IR	infra-red
ISPF	isopropenyl chloroformate
IPP	isopentenyl pyrophosphate
<i>iPr</i>	isopropyl
LRMS	low-resolution mass spectrometry
MDCK	Madin-Darby canine kidney cells
Me	methyl
MeCN	acetonitrile
MHB	Mueller Hinton Broth
MIC	minimum inhibitory concentration
min	minute
<i>M. luteus</i>	<i>Micrococcus luteus</i>
MOA	mechanism of action
m.p.	melting point
MRSA	methicillin-resistant <i>Staphylococcus aureus</i>
MSA	molecular surface area
MW	molecular weight
NA	not applicable
NBS	<i>N</i> -bromosuccinimide

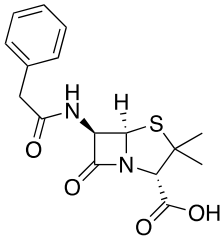
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
NOESY	nuclear Overhauser effect spectroscopy
<i>o</i>	ortho
o.n.	overnight
<i>p</i>	para
PAINS	pan-assay interference compounds
ppm	parts per million
PPTS	pyridinium <i>p</i> -toluenesulfonate
PSA	polar surface area
<i>p</i> -TsOH	<i>p</i> -toluenesulfonic acid
QSAR	quantitative structure-retention relationships
R_f	retention factor
r.t.	room temperature
RNA	ribonucleic acid
RNAP	RNA polymerase
RuPhos-Pd (G2)	2 nd generation RuPhos precatalyst, chloro(2-dicyclohexylphosphino-2',6-diisopropoxy-1,1'-biphenyl)[2-(2'-amino-1,1'-biphenyl)]palladium(II)
<i>S. aureus</i>	<i>Staphylococcus aureus</i>
sat.	saturated
SFC-MS	supercritical fluid chromatography mass spectrometry
SMD	solvation model based on density
sp.	species
Su	succinimide
tert	tertiary
TBAF	tetra- <i>n</i> -butylammonium fluoride
TFA	trifluoroacetic acid
THF	tetrahydrofuran
THP	tetrahydropyran
TLC	thin layer chromatography
TMSOTf	trimethylsilyl trifluoromethanesulfonate
TOF	time-of-flight
T ₃ P	propanephosphonic acid anhydride
Ts	tosyl (toluenesulfonyl)
UPPS	undecaprenyl diphosphate synthase
VRE	vancomycin-resistant <i>Enterococcus</i>
VT	variable temperature
w/v	weight per volume
w/w	weight per weight
XPhos	2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl
XPS	X-ray photoelectron spectroscopy

Chapter 1 Introduction

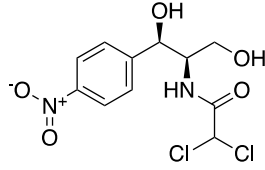
1.1 History of antibiotics

The term “antibiotics” was first used by microbiologist Selman Waksman and co-workers in 1942 to describe chemical substances produced by microorganisms that have an antagonistic effect on the growth of other microorganisms.¹ Traditional use of microorganisms for the management of microbial infections was documented in ancient China, Egypt, Greece and some other parts of the world.² The first scientific record of therapeutic antibiotic use dates back to 1899 when Emmerich and Löw explored the medicinal potential of *Pseudomonas aeruginosa* extracts to prevent the development of anthrax, an infectious disease in livestock caused by *Bacillus anthracis*.³

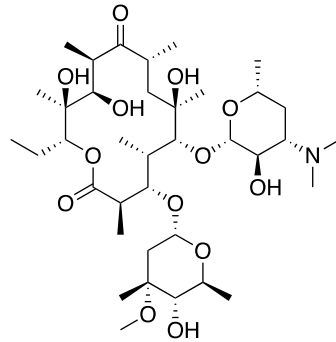
The modern era of antibiotics started with the serendipitous discovery of penicillin by Sir Alexander Fleming in 1928 from the fungus *Penicillium*,⁴ which was later found to be effective against bacterial infections caused by *Streptococcus pyogenes*, *Staphylococcus aureus*, and *Clostridium septique*.⁵ Systematic screening methods developed by Waksman in the 1940s, also known as the Waksman platform, screened well over 10,000 strains of complex soil bacteria, actinomycetes, and led to the discovery of many clinically relevant antibiotics, such as streptomycin, chloramphenicol, chlorotetracycline, erythromycin, vancomycin, rifamycin, polymyxin E, etc.³ Most antibiotics in clinical use today were discovered or derived from actinomycetes in this “golden era” of antibacterial drug discovery (Figure 1.1).⁶



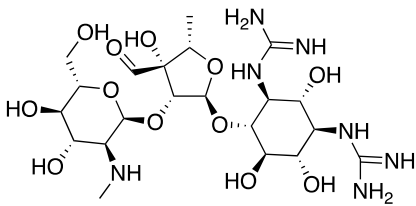
Penicillin G



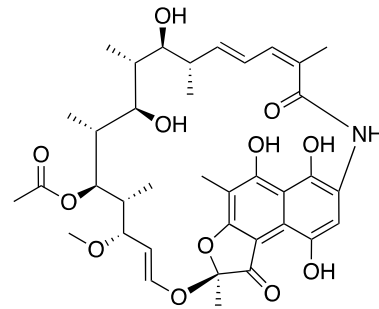
Chloramphenicol
(later succumbed to total synthesis)



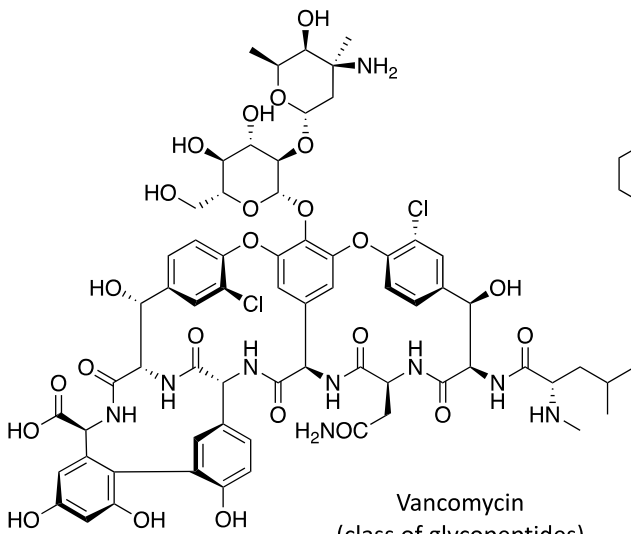
Erythromycin A
(class of macrolides)



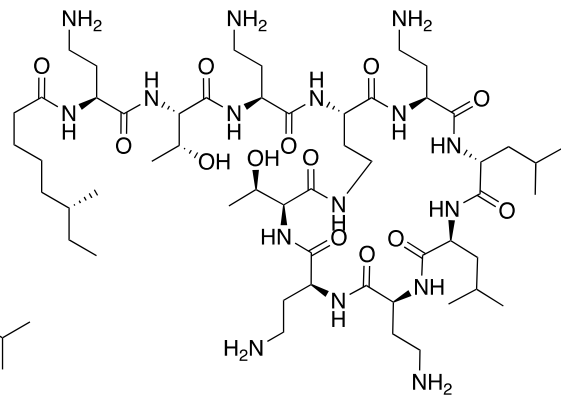
Streptomycin
(class of aminoglycosides)



Rifamycin SV



Vancomycin
(class of glycopeptides)



Polymyxin E
(also known as colistin)

Figure 1.1 Examples of antibiotics discovered in the “golden era”.

1.2 Classes of antibacterial drugs

Based on the different large-scale manufacturing processes, antibacterial drugs are categorised into natural product-derived, semi-synthetic and synthetic drugs. Following the discovery of antibiotics in the early 20th century, they were first mass-produced for consumption by fermentation of the relevant fungi or bacteria.⁵ In consideration of their therapeutic effectiveness and safety, semi-synthetic, the chemical modifications from existing scaffolds obtained from natural sources (Figure 1.3), and fully-synthetic approaches (Figure 1.4) produced many of the modern day antibacterial drugs including the entire class of beta-lactams (penicillins, cephalosporins, carbapenems, monobactams), tetracyclines, macrolides, metronidazole, trimethoprim, quinolones and oxazolidinones.³

Interestingly, the first antibacterial drug, arsphenamine (Salvarsan), was of synthetic origin.^{7,8} It was discovered by Paul Ehrlich and co-workers in 1907 through a systematic screening of chemical library for the treatment of syphilis, a sexually transmitted bacterial infection caused by *Treponema pallidum*.⁹ Systematic chemical modifications on the lead compound to expand the chemical library, followed by screening of activity on the relevant disease model, contributed to the discovery of neoarsphenamine (Neosalvarsan) in 1910, with increased aqueous solubility and reduced side effects than arsphenamine.³ Further investigations led to discovery of antibacterial sulfonamides, such as sulfamethoxazole.³

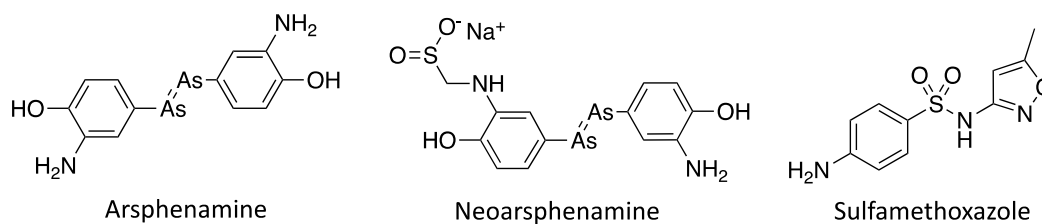


Figure 1.2 Structures of arsphenamine,^{8a} neoarsphenamine^a and sulfamethoxazole.

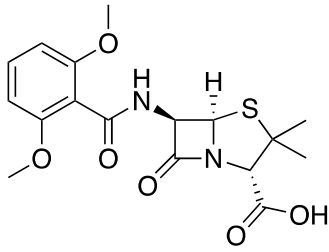
Through the discovery of salvarsan and neosalvarsan, Ehrlich introduced the ideas of a “magic bullet” - a chemical that selectively targets the disease-causing microorganisms leaving the host unaltered - and “chemotherapy” – historically with a broader meaning of the use of chemicals to treat any disease.⁵ From the initial discovery, a systematic approach of planned chemical modifications of an active lead compound for optimisation and screening of the synthesised library for activity also laid the foundation of modern pharmaceutical research and were also the guiding principles of this project.

Besides the different manufacturing processes, these clinically relevant antibacterial drugs may also be categorised based on their mechanisms of action,¹⁰ which are outlined in Table 1.1. Understanding the pharmacology of antibacterial drugs is important for understanding of antibacterial resistance as well as for discovery of novel antibacterial drugs.

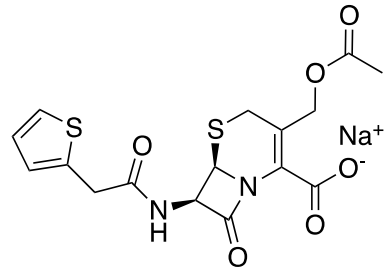
^a It was found in 2005 that the actual structure of arsphenamine was a mixture of trimer (cyclo-(RAs)₃) and pentamer (cyclo-(RAs)₅), where R is the 3-amino-4-hydroxyphenyl moiety.

Table 1.1 Overview of mechanisms of action (MOAs) of the antibacterial drugs.

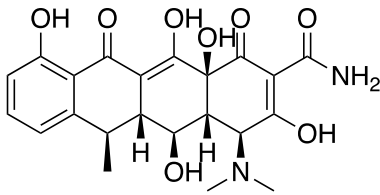
Mechanism of action		Class
Inhibition of cell wall synthesis		Penicillins Cephalosporins Carbapenems Monobactams Vancomycin
Disruption of bacterial cell membrane		Polymyxins
Inhibition of bacterial protein synthesis	By binding to 30S subunit of bacterial ribosome	Aminoglycosides Tetracyclines
	By binding to 50S subunit of bacterial ribosome	Macrolides Chloramphenicol Linezolid
	By disrupting bacterial DNA	Metronidazole
Inhibition of bacterial DNA synthesis	By inhibiting DNA topoisomerases	Fluoroquinolones
	By inhibiting folic acid synthesis	Sulfonamides Trimethoprim
Inhibition of bacterial RNA synthesis		Rifampin



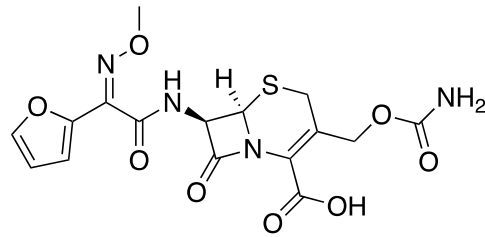
Methicillin
(class of penicillins)



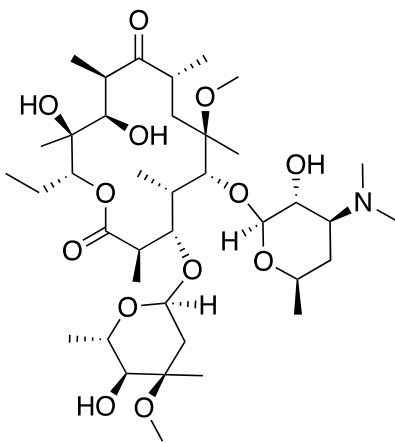
Cephalothin
(1st generation cephalosporin)



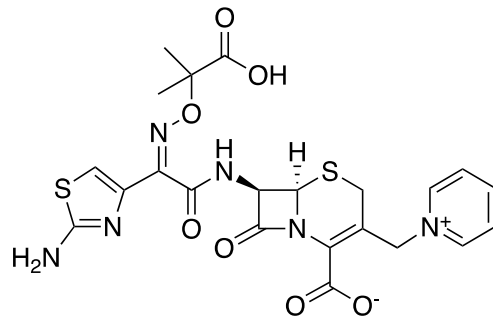
Doxycycline
(class of tetracyclines)



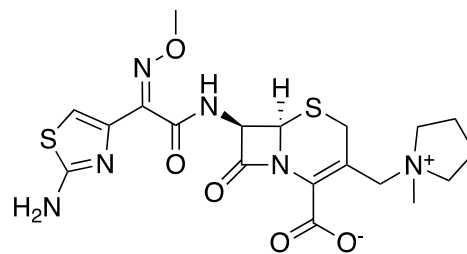
Cefuroxime
(2nd generation cephalosporin)



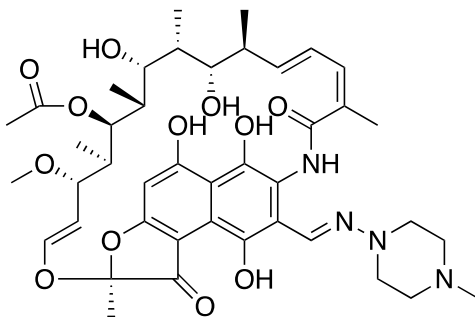
Clarithromycin
(class of macrolides)



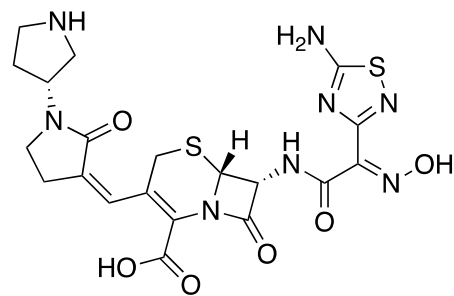
Ceftazidime
(3rd generation cephalosporin)



Cefepime
(4th generation cephalosporin)



Rifampin
(from rifamycin)



Ceftobiprole
(5th generation cephalosporin)

Figure 1.3 Examples of semi-synthetic antibacterial drugs.

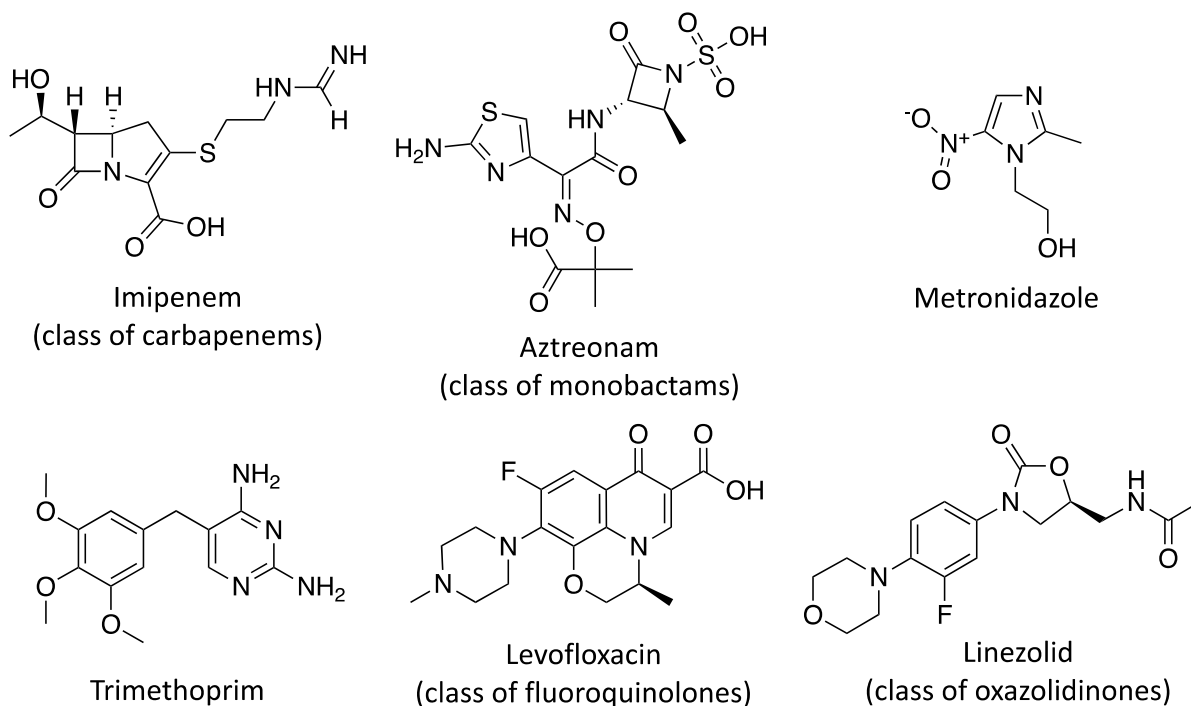


Figure 1.4 Examples of fully synthetic antibacterial drugs.

1.3 Antibacterial resistance

The development of antibacterial resistance is a natural phenomenon where pathogenic bacteria mutate, and under the selection pressure exerted by antibacterial drugs, some of them develop resistance and survive to pass on their resistance mechanisms.¹¹ Common resistance mechanisms include enzymatic degradation, target alterations, decreased bacterial uptake and over-expression of efflux pump proteins (Table 1.2).¹⁰

Table 1.2 Examples of antibacterial resistance mechanisms.

Resistance mechanism	Examples
Drug metabolism (enzymatic degradation)	Hydrolysis of beta-lactams by beta-lactamase Inactivation of aminoglycosides by acetylation or phosphorylation
Target alteration	Methylation of ribosome to inhibit macrolides binding Mutation of RNA polymerase to inhibit rifampin binding
Drug efflux	Tetracycline efflux pumps Multi-drug exporters

The problem of antibacterial resistance is not new, and has been in existence ever since mass consumption of penicillin began in the 1940s.¹² Whenever clinically relevant resistance occurred, the problem was dealt with by introducing new chemical modifications of existing antibiotics or introduction of new classes of antibiotics with novel mechanisms of actions.³ However, with an insufficient antibacterial pipeline as pharmaceutical companies strategically turned away from antibacterial drug research due to economic and regulatory obstacles, coupled with constant overuse and misuse of antibacterial drugs, resistance re-emerged as a major public health issue in the 21st century.¹³ The Centres for Disease Control and Prevention (CDC) in the United States declared in 2013 that we have entered the “post-antibiotic era” where the threat posed by antibacterial resistance is categorised as “urgent”, “serious” and “concerning” (Table 1.3).^{13,14} Mortality and morbidity due to resistant infections increase globally, with developing countries disproportionately impacted. In the US alone, each year more than 2.8 million people get an infection caused by resistant strains, resulting in more than 35,000 deaths.¹⁴ In Europe, antibacterial resistance is responsible for an estimated 33,000 deaths annually.¹³

Table 1.3 CDC assessment of antibacterial resistance threats.^{13,14}

Urgent threats	Carbapenem-resistant <i>Acinetobacter</i> and Enterobacteriaceae <i>Candida auris</i> <i>Clostridioides difficile</i> Drug-resistant <i>Neisseria gonorrhoeae</i>
Serious threats	Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA) Vancomycin-resistant <i>Enterococci</i> (VRE) Extended spectrum beta lactamase (ESBL)-producing Enterobacteriaceae Multidrug-resistant <i>Pseudomonas aeruginosa</i> Drug-resistant <i>Campylobacter</i> , <i>Candida</i> and <i>Shigella</i> Drug-resistant nontyphoidal <i>Salmonella</i> Drug-resistant <i>Salmonella</i> serotype Typhi Drug-resistant <i>Streptococcus pneumoniae</i> and <i>Tuberculosis</i>
Concerning threats	Erythromycin-resistant group A <i>Streptococcus</i> Clindamycin-resistant group B <i>Streptococcus</i>
Watch list	Azole-resistant <i>Aspergillus fumigatus</i> Drug-resistant <i>Mycoplasma genitalium</i> , <i>Bordetella pertussis</i>

1.4 Approaches to antibacterial resistance

To tackle the problem of antibacterial resistance requires a concerted effort from the scientific community, pharmaceutical companies, healthcare practitioners and regulatory bodies.¹⁵ Relevant to this project are the chemical approaches to antibacterial resistance, where new antibacterial drugs or drug combinations, with novel and/or multiple mechanisms of actions as well as resistance inhibition, are being explored.

Apart from efforts in new natural product isolation and semi-synthetic modifications by simple changes in functional groups, there are three traditional chemical approaches to generate new lead compounds: firstly, combinatorial chemistry in combination with target-based high-throughput screening (HTS); secondly, development of diverse chemical libraries through diversity-oriented synthesis and complexity-to-diversity (CtD) synthesis; and thirdly, modifications of previously identified antibacterial compounds through diverted total synthesis and biology-oriented synthesis.¹⁰

The first approach of target-based high-throughput screening (HTS) coupled with combinatorial chemistry has been extensively employed by industry to provide lead compounds.¹⁰ Combinatorial chemistry, often by cross-coupling of small molecules resulting in primarily sp²-hybridised compounds, allow rapid synthesis of large chemical libraries,¹⁶ which can be screened against a preselected protein target to identify hits in target-based HTS (with nM potencies).¹⁷ Although this approach is a fast route to identify structures, the hit rate is usually low and highly dependent on the size and diversity of chemical library used for screening.^{10,18} More recent HTS approaches have moved away from the numbers game of drug discovery and focused on screening lead-like compounds that represent pharmacophores known to exhibit desirable biological activity, many of which are simplified from natural products.¹⁹ It is however disappointing that HTS approach only generated one new chemical entity approved for clinical use between 1981 and 2010, sorafenib (Nexavar), an antitumor agent for the treatment of renal cell and hepatocellular carcinoma.²⁰ Interestingly though, a novel sorafenib derivative was found to exhibit high anti-MRSA potency without acute cytotoxicity towards human cells in 2015 by Chang et al.²¹ Wright and co-workers also noted in a review in 2016 that “despite new genomic tools, the ability to identify high-priority targets and innovation in HTS technologies, no new antibiotic drugs have emerged”.²² An alternative to HTS is fragment-based drug discovery (FBDD) which identifies low-molecular fragment hits (~150 Da) that bind to biologically important protein targets with high quality interactions despite having relatively weak binding affinity (mM potencies).¹⁹ Fragment-based hits have more controllable size, complexity and physical properties and can thus be optimised to drug leads with reduced attrition rates than HTS.¹⁹

In view of the limited diversity of chemical libraries generated by combinatorial chemistry, the second approach has seen extensive application in generating libraries of structurally complex and highly functionalised scaffolds.¹⁰ A diversity-oriented approach, originally introduced by Schreiber, uses simple chiral building blocks to generate libraries with high complexity through a few divergent transformations.^{16,23} This approach starts to move away from the relatively flat molecules generated from combinatorial chemistry.¹⁰ Complexity-to-diversity synthesis is another strategy to introduce diversity of chemical libraries. Starting with an abundant and highly functionalized structural scaffolds, complexity is generated by ring distortion through cleavage, rearrangement, or ring fusion.^{10,24-26}

The third approach centres on biologically active structures. In diverted total synthesis, introduced by Danishefsky and co-workers, a synthetic pathway to a previously identified active compound is designed that enables structural modifications of late-stage intermediates *en route* to the final compound.^{10,27,28} On the other hand, instead of total synthesis, biology-oriented synthesis, introduced by Waldmann and co-workers, classifies core scaffolds from structures with known biological activity and creates chemical libraries based on these scaffolds.²⁹

In summary, together with new compound isolation and semisynthetic modifications of existing antibacterial drugs, these chemical approaches allow rapid access to unexplored chemical space which in turn provides the potential for new antibacterial drug candidates to be discovered.

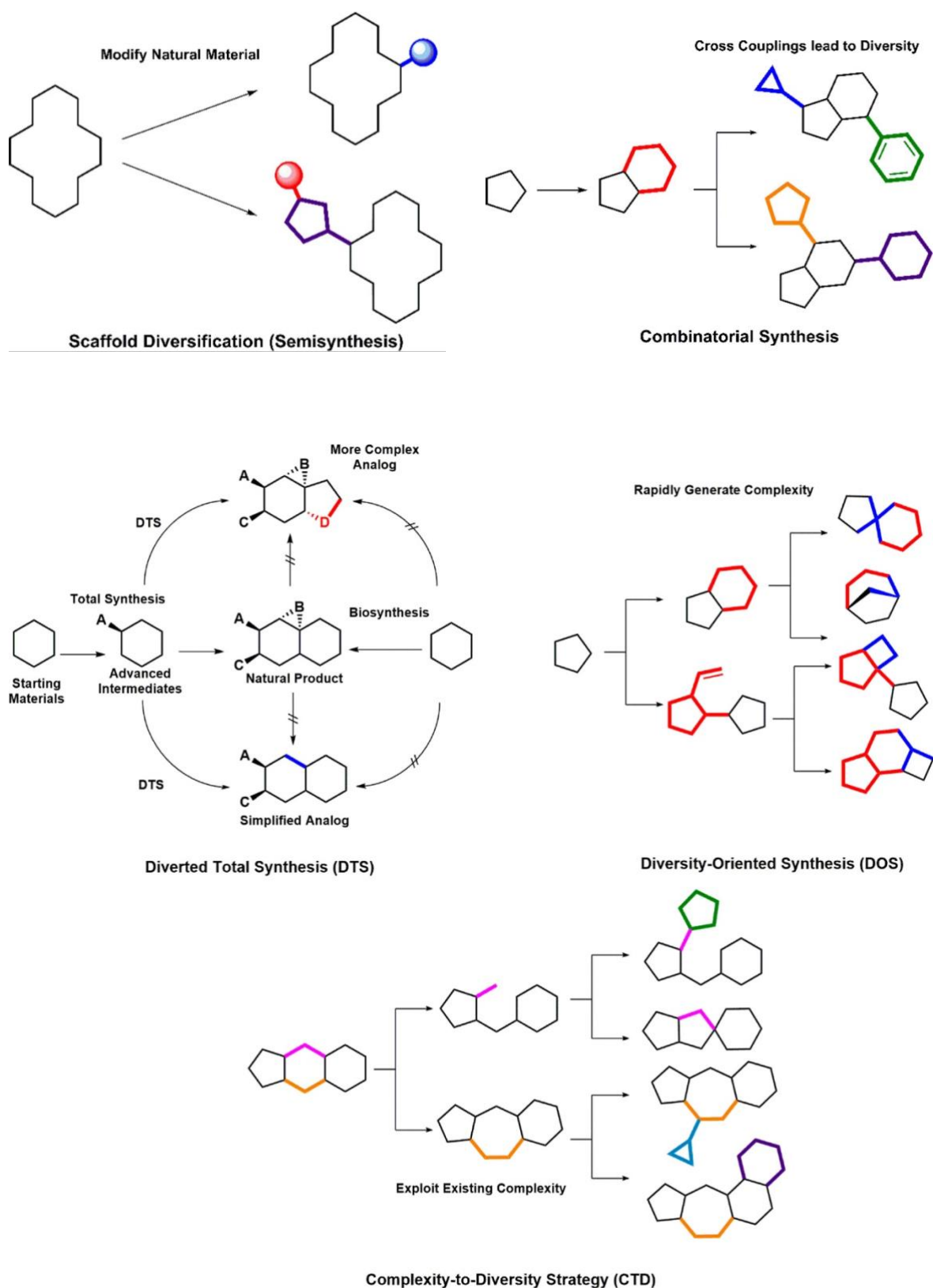


Figure 1.5 Overview of the chemical approaches to generate new lead molecules. Reprinted with permission from (Rossiter, S. E.; Madison, H.; Fletcher, M. H.; Wuest, W. M. *Chem. Rev.* **2017**, *117*, 12415–12474.). Copyright (2017) American Chemical Society.

1.5 Compound quality and drug-likeness

In the search for novel antibacterial drugs to combat the problem of antibacterial resistance, there are multiple criteria to take into consideration,³⁰ which are summarised in Table 1.4. Extensive optimisation has to be carried out to produce the best preclinical drug candidate with a balanced spectrum of drug-like properties. In this project, phenotypic screening of a synthesised chemical library was adopted where the overall effectiveness of chemical compounds was assessed and evaluated based on minimum inhibitory concentrations (MIC) against Gram-positive methicillin-resistant *Staphylococcus aureus* (MRSA) and Gram-negative *Escherichia coli*. Toxicity and safety in human use were assessed by IC₅₀ against mammalian cell lines (Hela, HeK-293, CaCo and MDCK). Evaluation of drug-likeness of the compounds was further aided by calculation of their physiochemical properties and comparison against the Lipinski's rule of five.³¹ It is worthy to note, though, that the Lipinski's rule of five is just a common guideline for orally administered drugs and there are many antibacterial drugs approved for clinical use that violate this rule. Evaluation of biological activity and physiochemical properties of the synthesised chemical libraries are detailed in Chapter 5.

Table 1.4 The concept of an ideal antibacterial drug.³⁰

Pathogen-specific criteria	Drug-specific criteria
Broad-spectrum bactericidal activity (Gram-positive, Gram-negative bacteria, <i>Mycoplasma</i> species with no cell wall, intracellular bacteria)	High oral availability (Lipinski's rule of five as a guideline) High aqueous solubility Low plasma protein binding
High potency (bactericidal at very low concentrations)	Accumulation in macrophages High permeability through bacterial cell membrane
Multiple bacterial targets in different metabolic pathways essential for bacterial survival	Safety in human use Low or no toxicity No teratogenic effects
Irreversible binding with bacterial targets, ruling out drug efflux	Not metabolised by human body No drug-drug interactions

1.6 Natural products as a source for novel antibacterial drugs

Natural products have been a crucial source of antibacterial agents since the golden era of antibiotics in the mid-20th century. Interest in natural products has waned over the course of antibacterial development.³² Increasing frequency of resistant bacterial infections has forced re-evaluation of natural products as a source to identify novel chemical skeletons with antibacterial activity for optimisation in drug development. In conjunction with high-throughput screening and the chemo-informatic approach, natural products continue to provide new chemical structures with high levels of antibacterial activity and these chemical scaffolds appear to occupy an unusual chemical property space.³³⁻³⁷ Many of the natural product derived structures are middle space molecules (MW 500 - 1500 Da) that readily provide high affinity interactions with protein targets yet can be optimised to achieve desirable oral absorption and cell permeability irrespective of molecular weight.³⁸ The common structural moiety of antibacterial natural products also allows a building-block approach which can be exploited for the rapid construction of new chemical systems.²⁹

1.6.1 Tetramic acids

In this project, the tetramate (pyrrolidine-2,4-dione)-containing natural products (Figure 1.6) with potent antibacterial activities (Table 1.5) are of interest.³⁹⁻⁴⁶ Identified modes of action of tetramate-based compounds include inhibition of bacterial kinase,⁴⁷ phosphatase,⁴⁷ RNA polymerase (RNAP)^{43,48-50} and undecaprenyl pyrophosphate synthase (UPPS),⁵¹⁻⁵³ membrane interference and depolarisation,⁵⁴⁻⁵⁶ metal accumulation and sequestration⁴⁷ as well as regulation of quorum sensing in bacteria.⁴⁷ These identified targets of tetramate-containing natural products mediate multiple and independent

bacterial metabolic pathways and have not yet been extensively exploited in clinically effective antibacterial drugs. Such natural product-inspired structures may also have added advantages for optimisation in drug discovery as they are more likely to be Lipinski-rule compliant and have low toxicity with increased molecular complexity by moving away from planar systems.^{57,58} The tetramic acid scaffold therefore serves as a promising starting point for antibacterial drug development.

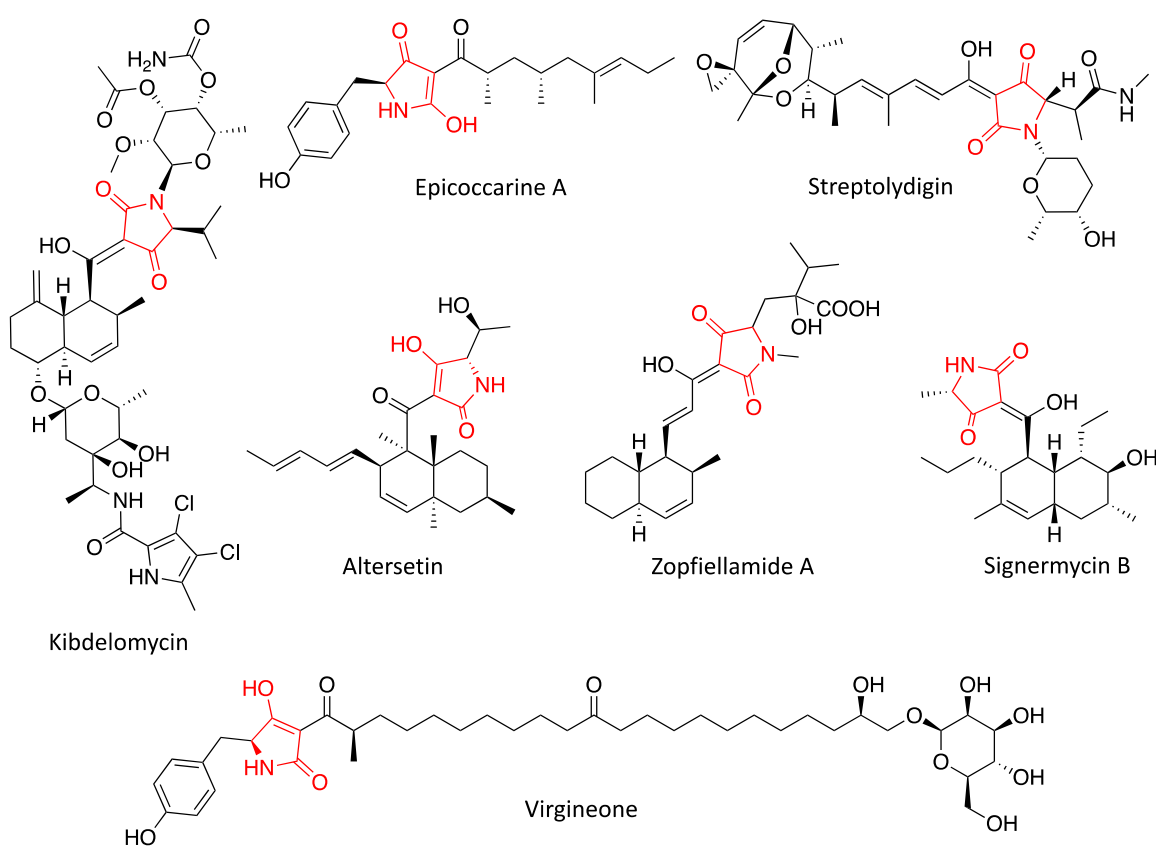


Figure 1.6 Examples of tetramate-containing natural products.

Table 1.5 Antibacterial activity of some tetramate-containing natural products.³⁹⁻⁴⁶

	Natural source	Effective against	MIC (µg/ml)
Epicoccarine A	<i>Epiccocum sp.</i>	<i>Mycobacterium vaccae</i>	6.25
Virgineone	<i>Lachnum sp.</i>	MRSA	7.2
Altersetin	<i>Alternaria sp.</i>	<i>Staphylococcus aureus</i> , <i>Streptococcus pneumonia</i> , <i>Streptococcus pyogenes</i> <i>Enterococcus faecalis</i>	0.12 - 2
Zopfiellamide A	<i>Zopfiella latipes</i>	<i>Arthrobacter citreus</i> , <i>Bacillus sp.</i> <i>Corynebacterium insidiosum</i> , <i>Micrococcus luteus</i> , <i>Mycobacterium phlei</i> , <i>Spreptomycetes sp.</i>	2 - 10
Signermycin B	<i>Streptomyces sp.</i>	<i>Bacillus subtilis</i> , MRSA, VRE	3.13 – 6.25
Kibdelomycin	<i>Kibdelosporangium sp.</i>	<i>Staphylococcus aureus</i> , <i>Streptococcus pneumonia</i> , <i>Enterococcus faecium</i> <i>Acinetovacter baumannii</i>	0.125 - 1
Streptolydigin	<i>Streptomyces lydicus</i>	<i>Clotridium sp.</i> <i>Streptococcus sp.</i>	Lowest at 0.04

1.6.2 Metal-chelating properties of tetramates

Note-worthy is that tetramate-containing natural products were reported to exhibit metal-binding properties.^{47,59} Depending on the nature of the coordinated metal ions, either an increase or a decrease of biological activity can be observed for metal-bound compounds when compared with their metal-free parent compounds.^{47,60} Moreover, polarity and aqueous solubility of these compounds can be greatly influenced by metal chelation.⁶⁰ The stability of metal coordination is another crucial factor affecting biological activity of the metal-bound compounds.⁴⁷ Tetramate-based compounds were reported to display “hard” *O,O*-chelating ligand systems that bind strongly to “hard” metal ions such as Mg(II) or Fe(III) (Figure 1.7).⁴⁷ Various other metal chelates have also been reported such as Na(I), K(I), Ca(II), Ba(II), Al(III), Mn(II), Co(II), Ni(II), Cu(II), Zn(II),

Ga(III), Ru(III), Rh(II), Cd(II), La(III), Tb(III), Pt(II) and Hg(II).⁶¹ It has been suggested that metal-binding tetramates could play an important role in metal sequestration, and scavenging and deprivation of essential metal ions from bacterial cells, giving rise to the idea of formulating tetramate-based prodrugs that act as “Trojan horses”.⁴⁷ On the other hand, metal chelation might also contribute to the off-target effects of tetramates; controlling their metal binding properties help reduce potential promiscuous interactions with proteins during target-based screening so that tetramate-based structures do not fall into the category of pan-assay interference compounds (PAINS) which waste efforts in drug optimisation.⁶²

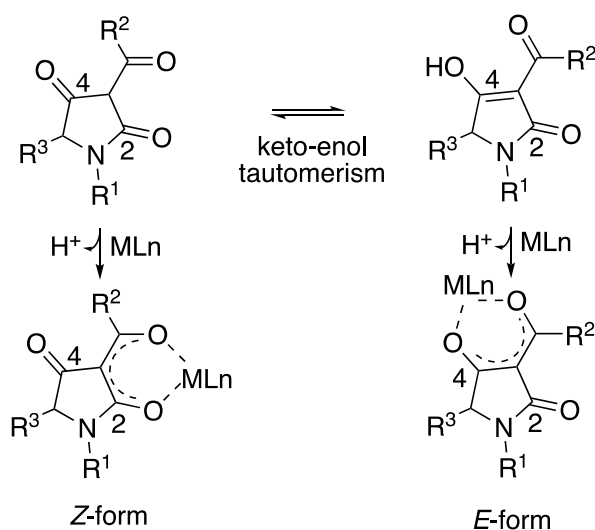


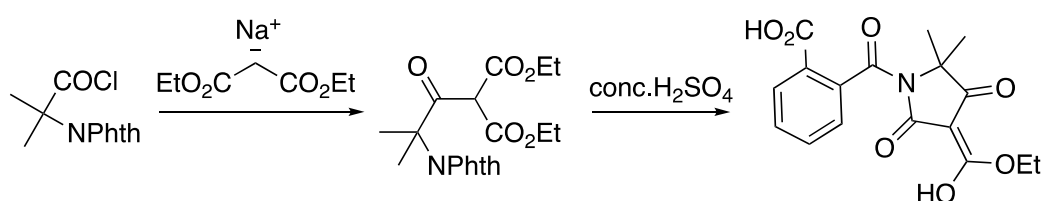
Figure 1.7 Models of metal-chelation in tetramic acids. Adapted with permission from Expert Opinion on Drug Discovery (Dandawatea, P.; Padhyeb, S.; Schobertc R.; Biersack, B. *Expert Opin. Drug Discovery* **2019**, *14* (6), 563-576). Copyright (2019) Taylor & Francis.

1.7 Synthesis of tetramate-based compounds

Various methodologies for synthesis of tetramate-based derivatives have been reported.⁵⁹ Most of the conventional synthetic approaches were dedicated to the synthesis of 3-acyltetramic acids, the most commonly found variant in natural products and thus of high biological relevance.⁵⁹ The synthetic methodologies for 3-acyltetramic acids are broadly categorised by their starting materials into 1) synthesis from active methylene compounds, 2) synthesis from diketene in Lacey's modified Dieckmann cyclisation, 3) synthesis from activated cyclic starting materials and 4) synthesis from phosphorous ylides. 3-Unsubstituted tetramic acids can be derived from 3-acyltetramic acids by decarboxylation⁵⁹ or from Meldrum's acid in Jouin's procedure, which can be further functionalised by C-3 acylation for diversification.

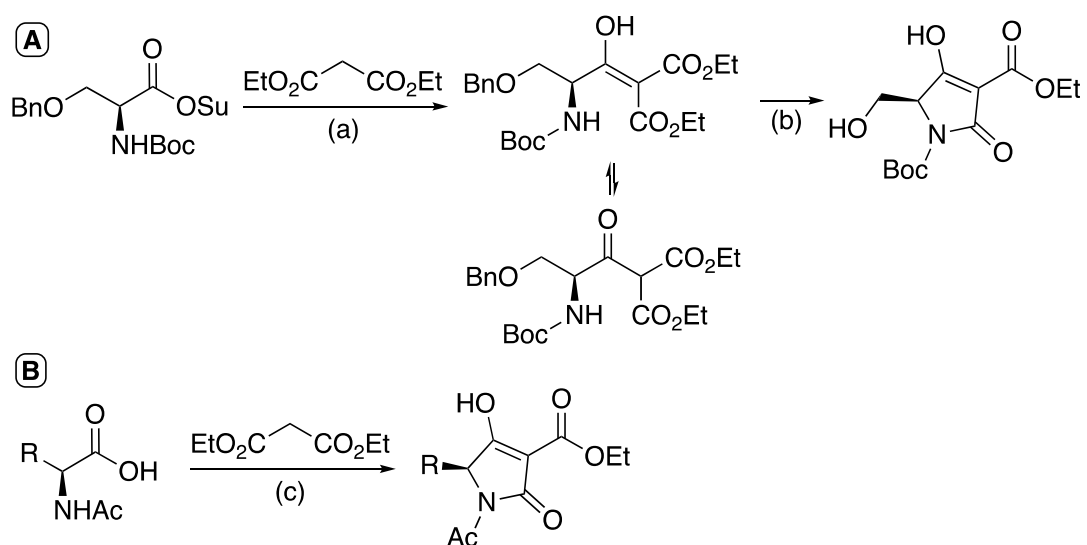
1.7.1 Synthesis from active methylene compounds

The first synthesis of a tetramic acid derivative was reported by S. Gabriel in 1914 using diethyl sodiomalonate and phthalimidoisobutyryl chloride, whose product on treatment with concentrated sulphuric acid cyclised to the 3-ethoxycarbonyl tetramate acid (Scheme 1.1).⁶³⁻⁶⁵



Scheme 1.1 Synthesis of a tetramic acid derivative by S. Gabriel.⁶³⁻⁶⁵

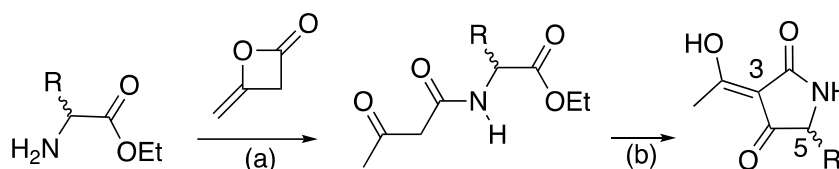
Preparation of tetramic acid derivatives from similar active methylene compounds (β -ketoesters or diesters of malonate acids) was advanced by Markopoulou et al.⁶⁶⁻⁷⁰ in the early 1980s. The key step in the synthetic protocol was a C-acylation between a protected, activated α -amino acid and diethyl malonate. The resulting acylaminoacetyl derivative cyclised under acidic or basic conditions (10% HCl or 2N NaOH) to give the corresponding tetramate acid (Scheme 1.2). The common acylating agents were DCC and *N*-hydroxysuccinimide (Scheme 1.2 A)⁶⁶⁻⁶⁹ or 1-hydroxybenzotriazole (HOBt) (Scheme 1.2 B),⁷⁰ which were able to give clean and enantiopure products upon cyclisation in high yield with ee up to 99% by chiral HPLC.



Scheme 1.2 Synthesis of 3-acyltetramic acid derivatives by Markopoulou et al.⁶⁶⁻⁷⁰
Reagents and conditions: (a) NaH, THF, 0 °C to r.t., 1 h, Su = succinimide; (b) 2N NaOH, MeOH, r.t., 2 h; followed by H₂, Pd/C, r.t., 1 h; (c) HOBt, DCC, NaH, THF, 'one-pot', 0 °C, 1 h, warm to 3-5 °C, o.n.

1.7.2 Lacey's modified Dieckmann cyclisation

A versatile and convenient route to tetramic acid is the Lacey's modified Dieckmann cyclisation.⁵⁹ In 1954, Lacey reported a two-step synthesis of 3-acyltetramic acid from readily available amino acids, which upon treatment of diketene, gave *N*-acetoacetyl- α -amino esters.⁷¹ The synthetic route was followed by a Dieckmann cyclisation - a base-induced intramolecular Claisen condensation - with sodium ethoxide to give the 3-acyltetramic acid (Scheme 1.3).⁷¹

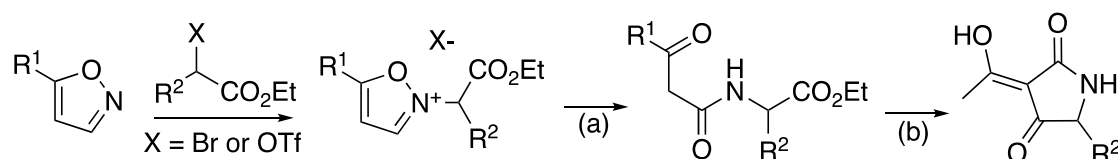


Scheme 1.3 Lacey's modified Dieckmann cyclisation.⁷¹ *Reagents and conditions:* (a) EtOH, 5 °C to r.t., 1 h; (b) NaOMe, MeOH, benzene, reflux for 3 h and then cooled to r.t., o.n.

Many modifications of Lacey's Dieckmann cyclisation have been published since then.⁵⁹ The variation at C-3 was restricted by the availability of the substituted diketenes. To overcome this limitation, Isowa and Ohta reported the use of ethoxycarbonylacetyl chloride instead of the diketene;⁷² Mulholland et al. used malonic ester chlorides in replacement.⁷³ Another limitation of Lacey's modified Dieckmann cyclisation was the potential racemisation at C-5 due to deprotonation of the acidic hydrogen under basic conditions. A significant advancement of the approach was reported by Ley et al. in 1992 to achieve enantioselective synthesis of 3-acyltetramic acids.^{74,75} The key modification for stereo-control was the use of milder base, TBAF or KO^tBu for a shorter reaction time.

1.7.3 Synthesis from activated cyclic starting materials

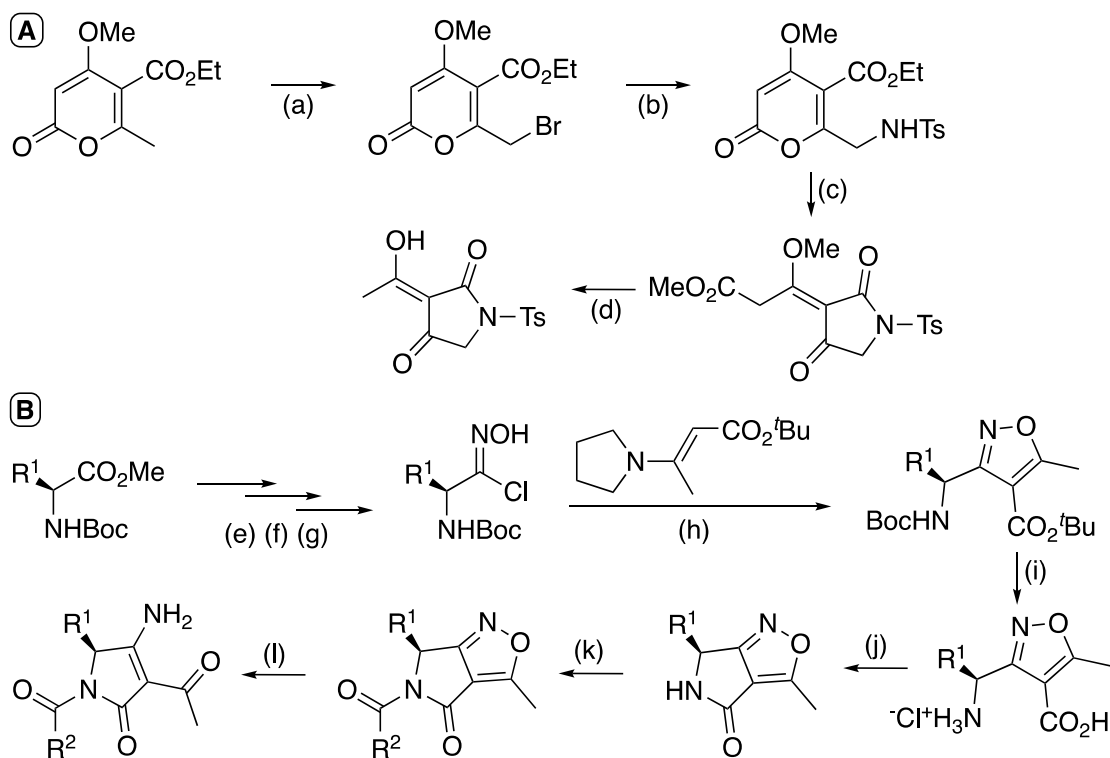
Based on Lacey's protocol, DeShong et al. devised a synthetic route from a substituted isoxazole, which produced a β -ketoamide by alkylation and subsequent fragmentation.^{76,77} The intermediate then cyclised under the basic condition of Lacey's procedure to yield a 3-acyltetramic acid (Scheme 1.4).^{76,77}



Scheme 1.4 Synthesis of 3-acyltetramic acids from isoxazoles by DeShong et al.^{76,77}

Reagents and conditions: (a) Et₃N, DCM/H₂O, r.t., 5 h; (b) KO^tBu, *t*-BuOH, reflux, 5 h.

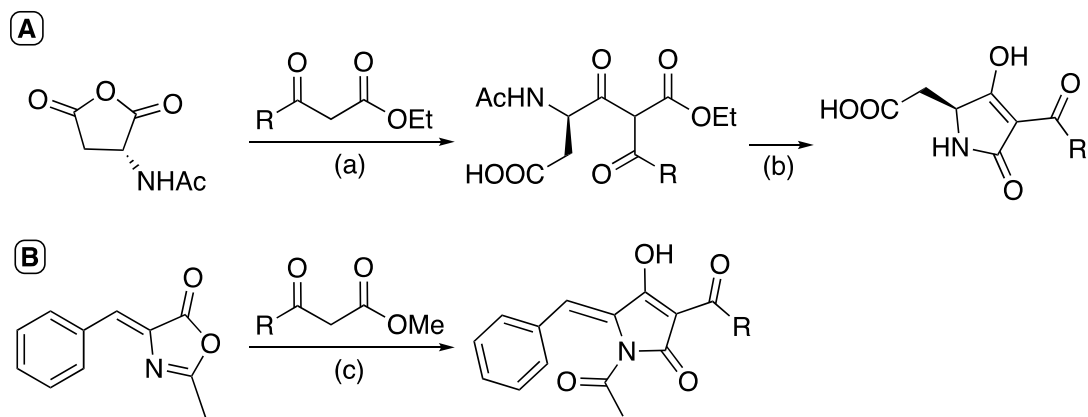
In addition, Jones et al. developed two synthetic protocols from cyclic starting materials, one from pyrone (δ -lactone) precursors (Scheme 1.5 A)⁷⁸ and the other from active isoxazolecarboxylic esters (Scheme 1.5 B) using a cycloaddition approach^{79,80} to access 3-acyltetramic acids.



Scheme 1.5 Synthesis of 3-acyltetramate acids from cyclic starting materials by Jones et al.⁷⁸⁻⁸⁰ *Reagents and conditions:* (a) NBS, AIBN, CCl₄, reflux; (b) NaNHTs, THF, 20 °C; (c) NaOMe, MeOH, reflux; (d) NaOH, aq., 25 °C; (e) DIBAL-H, toluene, -78 °C; (f) NH₂OH.HCl, NaOAc, EtOH aq., 70 °C; (g) *N*-chlorosuccinimide, CHCl₃, reflux, 18 h; (h) Et₃N, CHCl₃, reflux; (i) TFA, 20 °C, followed by 2M HCl; (j) T₃P, EtOAc, 0 to 20 °C, 17 h or EDCI, *N*-hydroxysuccinimide, DMF/DCM, 20 °C, 17 h; (k) BuLi, THF, -78 °C, R²COCl; (l) H₂ (1atm), Pd/C, EtOH or Mo(CO)₆, aq. MeCN followed by 2M HCl.

More recently, Igglessi-Markopoulou et al. reported synthesis of 3-acyltetramic acids from cyclic (*S*)-*N*-acetyl-aspartic anhydride as chiral building blocks (Scheme 1.6 A).⁸¹ Another protocol by the same group utilised cyclic 2-methyl-4-benzylidene-5(4H)-oxazolone to prepare 5-benzylidene tetramic acid in just one step (Scheme 1.6 B).⁸² The *N*-acetyl group was found to be crucial for biological activities.⁷⁹ Stachel et al. also

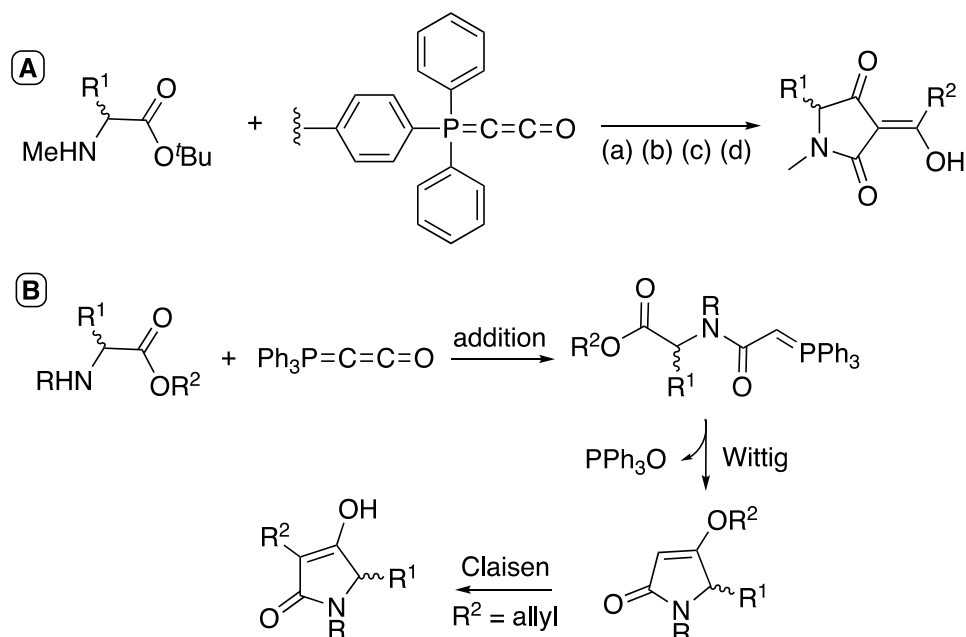
reported the synthesis of *N*-benzoyl 5-arylidene tetramic acids from a similar 2-phenyl oxazolone.⁸³



Scheme 1.6 Synthesis of 3-acyltetramate acids from cyclic starting materials by Markopoulou et al.^{81,82} *Reagents and conditions:* (a) NaH, THF, 0 °C to r.t., 1 h; (b) NaOEt/EtOH, r.t., o.n.; (c) NaH, THF, 0 °C to r.t., 2 h.

1.7.4 Synthesis from phosphorous ylides

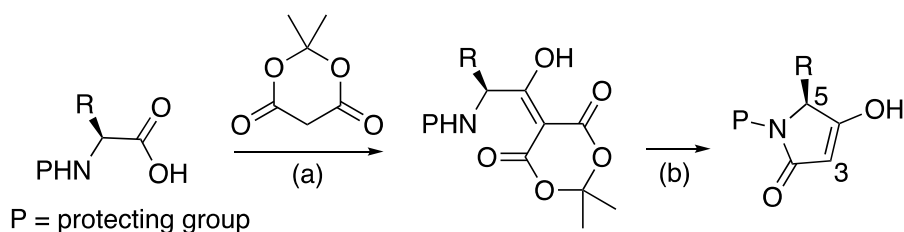
In 2004 and 2005, Schobert et al. reported the synthesis of 3-acyltetramic acids from solid supported (triphenylphosphoranylidene)ketene (Ph₃PCCO), which was involved in a Wittig-type cyclisation under mild conditions (Scheme 1.7 A).⁸⁴⁻⁸⁶ The synthetic methodology followed the initial work published by the same group in 1996 to access 4-alkoxytetramic acids from α -amino carboxylic esters by an addition/Wittig olefination/Claisen rearrangement sequence (Scheme 1.7 B).⁸⁷



Scheme 1.7 Synthesis of 3-acyltetramate acids from phosphorous ylides by Schobert et al.⁸⁴⁻⁸⁷ *Reagents and conditions:* (a) THF, 60 °C, 12 h; (b) TFA, r.t., 3 h.; (c) R^2COCl , BF_3OEt_2 , microwave, 100 °C, 45 min; (d) MeOH, reflux, 2 h.

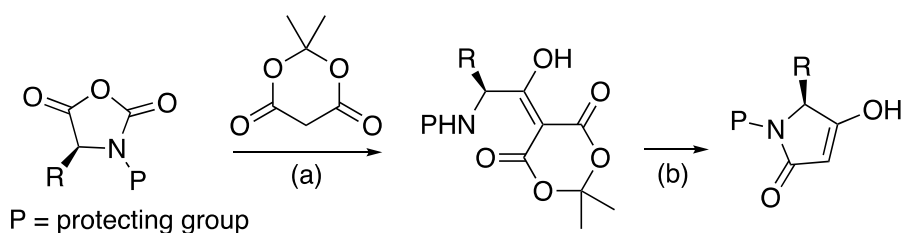
1.7.5 Preparation of 3-substituted tetramic acids: synthesis from Meldrum's acid

A mild procedure was introduced in 1987 as Jouin's method for the preparation of 3-unsubstituted tetramic acid analogues.⁸⁸ A C-acylation reaction was achieved between the appropriate α -amino acids and Meldrum's acid by isopropenyl chloroformate (ISPF), using 4-dimethylamino-pyridine (DMAP) as the catalyst (Scheme 1.8).⁸⁸ Alternative acylating agents to ISPF include 1,1-carbonyldiimidazole (CDI) and N,N' -dicyclohexylcarbodiimide (DCC).^{89,90} Heating the resulting ester in acetonitrile or ethyl acetate led to the corresponding tetramic acid with concomitant formation of acetone and carbon dioxide (Scheme 1.8).⁸⁸



Scheme 1.8 Synthesis of 3-unsubstituted tetramate acids from Meldrum's acid by Jouin's protocol.⁸⁸ *Reagents and conditions:* (a) ISPF, DMAP, DCM, -5 °C, 2.5 h; (b) MeCN or EtOAc, reflux, 30 min.

An interesting modification of Jouin's method was reported by Martinez et al. in 1994, using a cyclic starting material, protected urethane-*N*-carboxyanhydride (Fmoc, Boc or carboxybenzyl protected) to prepare *N*-protected 3-unsubstituted chiral tetramic acids (Scheme 1.9).⁹¹

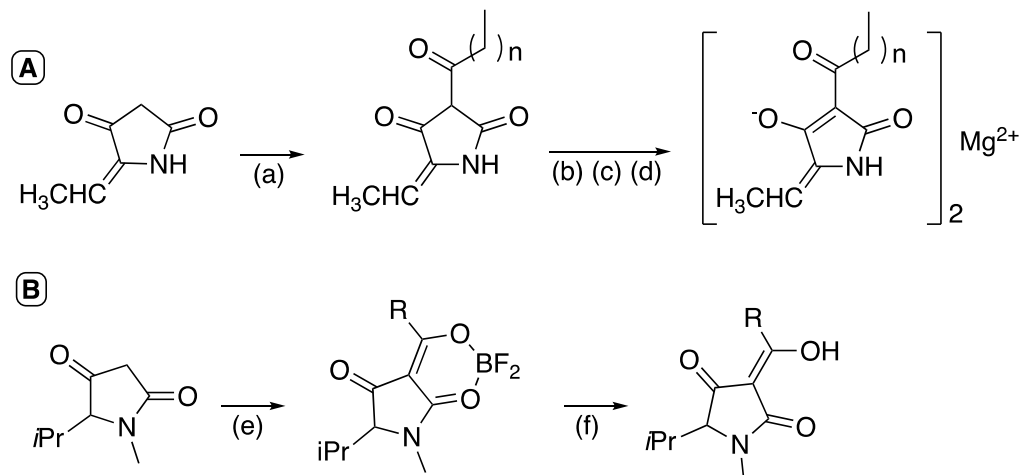


Scheme 1.9 Synthesis of 3-unsubstituted tetramate acids by Martinez et al.⁹¹ *Reagents and conditions:* (a) Et₃N, THF, r.t., < 10 min; (b) MeCN or EtOAc, reflux, 30 min.

1.7.6 C-3 acylation of unsubstituted tetramic acids

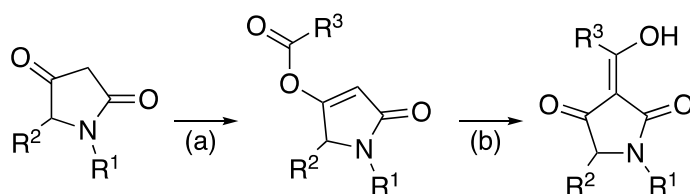
The synthesis of 3-acyltetramic acids from C-3 acylation of unsubstituted tetramate core was first employed by Kohl et al. in 1974 using an acyl chloride in presence of boron trifluoride diethyl etherate (BF₃·OEt₂) (Scheme 1.10 A).⁹² This procedure was further investigated by Jones and coworkers in 1983, isolating tetramic acids in boron-difluoride

complexes from neutral work-up conditions in good yield (50-78%), which upon treatment of methanol yielded tetramic acids (Scheme 1.10 B).⁹³



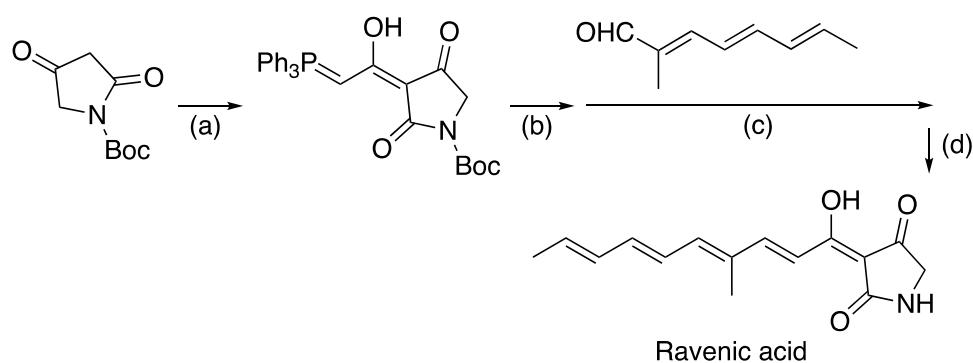
Scheme 1.10 C-acylation of unsubstituted tetramic acids with a Lewis acid.^{92,93} *Reagents and conditions:* (a) $\text{CH}_3(\text{CH}_2)_n\text{COCl}$, $\text{BF}_3 \cdot \text{OEt}_2$, $n = 4, 6$; (b) 0.1 N NaOH; (c) MgCl_2 ; (d) NaOAc, acetone; (e) RCOCl , $\text{BF}_3 \cdot \text{OEt}_2$, 80 °C; (f) MeOH, 25-65 °C.

In 1987, Yoshii et al. described a mild base-induced ‘one-pot’ acylation route, where the tetramic acids were acylated at O-4 and the resultant tetramate O-4 esters exposed directly to triethylamine (Et_3N) and rearranged to the 3-acyltetramic acids (Scheme 1.11).^{63,94}



Scheme 1.11 ‘One-pot’ C-acylation of unsubstituted tetramic acids by Yoshii et al.^{63,94} *Reagents and conditions:* (a) $\text{R}^3\text{CO}_2\text{H}$, DCC, DMAP, DCM, 0 °C to r.t., 1.5 h.; (b) Et_3N , DCM, 0 °C for 10 min, warm to r.t., 4.5 h.

An interesting procedure of C-3 acylation was reported by Schobert et al. in 2010 following their investigations of the use of phosphorous ylides for the synthesis of tetramic acid derivatives.⁹⁵ A 3-unsubstituted tetramic acid core, with a C-3 acidic hydrogen, reacted with Ph_3PCCO to produce the corresponding 3-(triphenylphosphoranylidene)acetyl derivatives, which upon deprotonation and activation by KO^tBu in THF, reacted with an aldehyde to yield the target 3-acyl tetramic acid (Scheme 1.12).^{95,96} The protocol was used for the total synthesis of many tetramate-based natural products such as ravenic acid,⁹⁵ 5*R*-reutericyclin,⁹⁷ penicillenols B₁, B₂,⁹⁸ C₁, and an isomer of methiosetin.⁹⁹

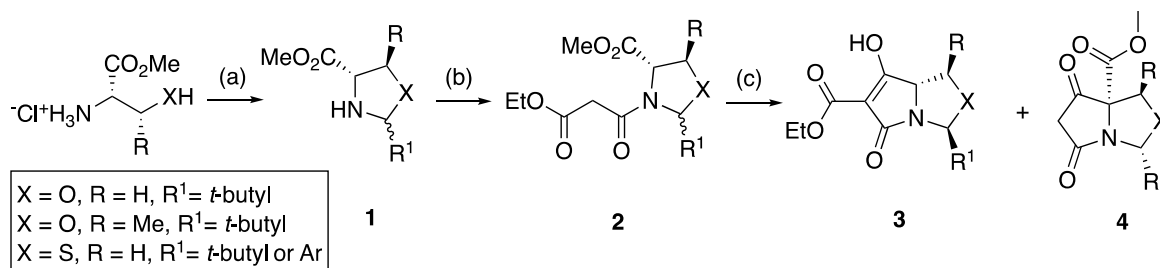


Scheme 1.12 C-acylation of unsubstituted tetramic acids with phosphorous ylides by Schobert et al.^{95,96} *Reagents and conditions:* (a) Ph_3PCCO , dry THF, reflux, 16 h; (b) KO^tBu , dry THF, reflux, 20 min; (c) dry THF, reflux, 6 h; (d) TFA, DCM, r.t., 30 min.

1.7.7 Synthesis of tetramic acid derivatives in the Moloney group

One of the most interesting and well-established methods for the preparation of chiral tetramic acids is Moloney's method. Using the Self Regeneration of Stereocentre concept developed by Seebach,^{100,101} the Moloney group has demonstrated rapid access to enantiopure bicyclic tetramate core **3** and **4** from serine, threonine and cysteine (Scheme

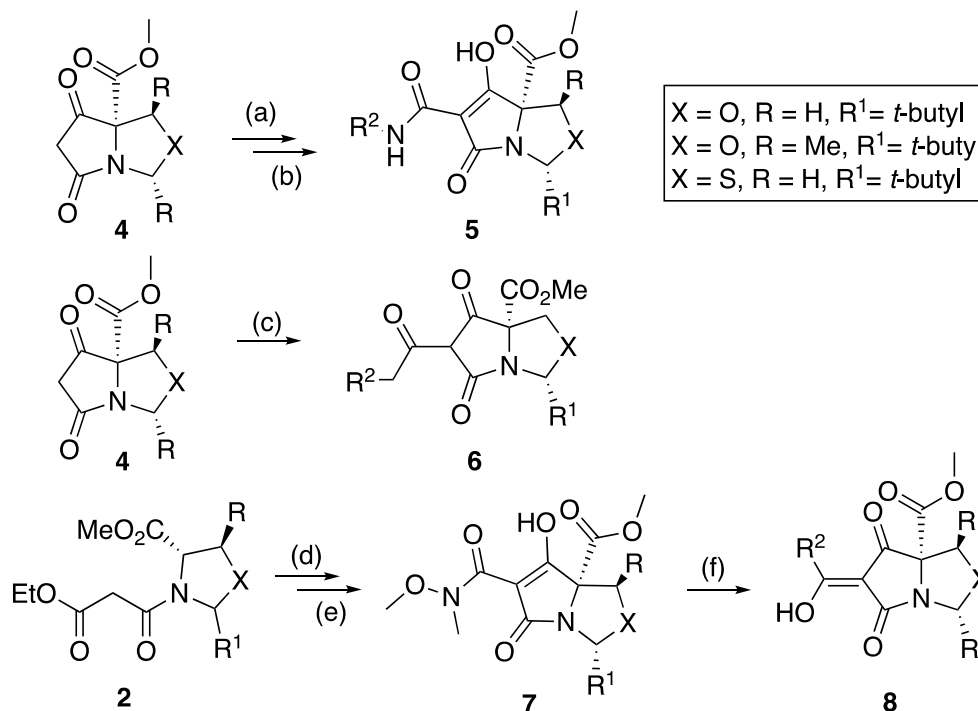
1.13).¹⁰²⁻¹⁰⁹ This synthesis relies on highly chemoselective and diastereoselective Dieckmann cyclisation of *N*-acylated oxazolidines or thiazolidines to give tetramates in high yield and enantioselectivity. Although the oxazolidine system is limited to pivaldehyde (*t*-BuCHO) as the initial condensing agent, the thiazolidine system allows greater variations at the C-2 position, for which condensation with aromatic aldehydes is possible.¹⁰⁹ Interestingly, the reduction in steric demand of the C-2 substituents in the thiazolidine system results in a change in the selectivity of the Dieckmann cyclisation – giving tetramate ester **3** as the major product instead of tetramate **4** in the oxazolidine system.



Scheme 1.13 Synthesis of the tetramate core by the Moloney group.¹⁰²⁻¹⁰⁹ *Reagents and conditions:* (a) R¹CHO, Et₃N, petrol 40/60, reflux, 18 h; (b) mono-ethyl malonate, DCC, DMAP, DCM, 0 °C to r.t., 18 h; (c) KO^tBu, THF, reflux, 3 h.

Although the tetramate core has no or little intrinsic antibacterial activity, it can be functionalised to mimic features of tetramate-containing natural products. Various methods to functionalise the C-7 position have been exploited by the Moloney group: C-7 carboxamides are synthesised from **3** by transamidation reactions with the corresponding primary amides;^{48,110} preparation of acyltetramates is possible from **4** with the corresponding carboxylic acid and stoichiometric amount of DCC-DMAP (Scheme 1.14).¹¹¹

Elaborate functionalisation at C-7 was also reported with the use of Weinreb amides and subsequent Grignard reactions (Scheme 1.14).¹¹²



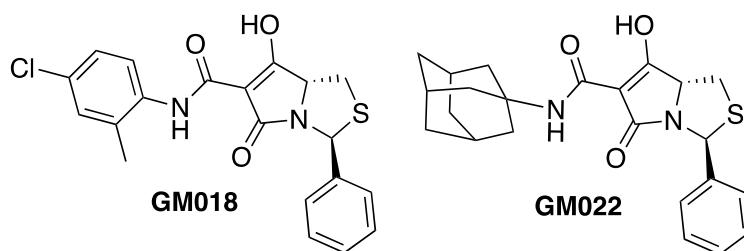
Scheme 1.14 Functionalisation of the tetramate core by the Moloney group.^{48,110-112}

Reagents and conditions: (a) Butyl chloroformate, DMAP, DCM, r.t., o.n.; (b) R²NH₂, toluene, reflux, 4 h; (c) R²CH₂COOH, DCC, DMAP, DCM, r.t., 18 h; (d) methoxy(methyl)carbamic chloride, pyridine, MgBr₂, DCM, r.t., 24 h; (e) DBU, THF, 24 h; (f) R²MgBr, THF, -15°C, 2 h.

1.8 Aim of the project

Previous work in the Moloney group has shown that functionalised cysteine-derived tetramates possess excellent antibacterial activities (Table 1.6).¹¹⁰ A major setback with these compounds was that the *in vitro* activity diminished when tested in the presence of blood (Table 1.6).

Table 1.6 Bioactivity of carboxamide tetramic acids prepared by the Moloney group.



MIC against MRSA ($\mu\text{g/ml}$)	3.91 - 7.81	0.98 - 3.91
In the presence of blood	31.25 - 250	7.81 - 15.63

It was hypothesised that the reduction of bioactivities in the presence of blood was associated with plasma protein binding and/or metal chelation. This project thus aimed to design and synthesise a chemical library of tetramates with decreased plasma protein binding and/or reduced metal chelation and to evaluate them for antibacterial activity. This project would focus on cysteine-derived tetramate system as preliminary biological results showed marked improvement of antibacterial activity over the corresponding systems derived from serine or threonine. Chapter 2 explores the possibility of incorporating polar heterocycles at C-2 to increase aqueous solubility and decrease plasma protein binding. Chapters 3 and 4 describe approaches to alter the metal chelating properties of tetramate systems by chemical modifications at C-6, C-9 carbonyl groups and C-5 esters. The assessment and biological evaluation of the chemical library (Figure 1.8) are summarised in Chapter 5.

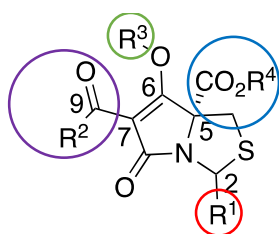


Figure 1.8 Design of target compounds by functionalisation of tetramate core.

Chapter 2 Approaches to increase polarity of cysteine-derived tetramate systems

“Without convincing evidence to the contrary, drugs should be made as hydrophilic as possible without loss of efficacy.”¹¹³

— Corwin Hansch, 1987

This chapter describes the synthesis of cysteine-derived tetramate analogues with functionalisation at C-2 and C-7 (Figure 2.1). The aim of functionalisation was to incorporate polar heterocycles to the tetramate system to increase compound polarity and decrease plasma protein binding. The chemoselectivity and stereoselectivity of the synthetic pathway is discussed in comparison with pathways leading to other tetramate systems derived from oxazolidine and/or with different condensing species. The synthesised tetramate analogues are further analysed in terms of their characterisation, metal chelation property and biological activity. Some content in this chapter have been reproduced with permission from (Zhang, R.; Li, X.; Genov, M.; Pretsch, A.; Pretsch, D.; Moloney, M.G., *J. Org. Chem.* **2020**, *85* (19), 12393–12407). Copyright (2020) American Chemical Society.

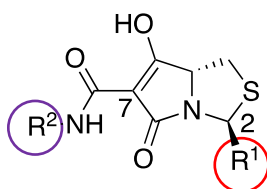


Figure 2.1 Cysteine-derived tetramate analogues with C-2 and C-7 functionalisation.

2.1 Introduction

As described in Chapter 1, functionalised cysteine-derived tetramic acids exhibited excellent antibacterial activity against MRSA but their *in vitro* activities diminished when tested in the presence of blood (Table 1.6, page 28, *vide supra*). It was hypothesised that plasma protein binding was one key factor associated with the reduction in bioactivity. In blood, drugs bind with plasma proteins usually in a reversible manner and an equilibrium exists between bound and free drug forms.^{114,115} Without the presence of a specific drug transporter, it is the free unbound drug molecules that are transported across cell membrane barrier and exert biological action.^{114,115} The extent and affinity of plasma protein binding thus affect permeability of drug molecules across bacterial cell membrane, impacting effective concentration and hence potency.

In plasma, there are two major proteins, human serum albumin (HSA) and α_1 -acid glycoprotein (AGP), present in relatively high concentrations and reported to bind with a variety of drugs with considerable affinity.¹¹⁶⁻¹¹⁸ HSA, with the highest concentrations of 5 - 7.5×10^{-4} mol/L in plasma, demonstrates a preference for binding acidic drugs with relatively high lipophilicity.^{117,118} AGP, with a significantly lower concentration of 0.9 – 2.2×10^{-5} mol/L, binds with basic and neutral drugs.¹¹⁸ Lipoproteins also exhibit drug-protein binding but to a lesser extent.^{117,118} Given its relative abundance and the fact that tetramate derivatives have an acidic C-6 enol group, HSA is the main protein of relevance in the discussion of plasma protein binding of tetramate analogues. Indeed, cysteine-derived tetramate carboxamides were found to be 6 times less potent when tested in presence of HSA in whole-cell antibacterial assays.¹¹⁰ Complex binding modes have been reported between various drugs and HSA – with two primary binding sites of largely

hydrophobic pockets (IIA and IIIA) and several other secondary and allosteric sites (Figure 2.2).¹¹⁹⁻¹²¹ Based on quantitative structure-retention relationships (QSAR) models for drug binding affinities, binding to HSA is favoured by hydrophobicity of compounds with good correlations.^{122,123} Hence in general, an increase in ligand polarity is associated with a decrease in HSA binding.

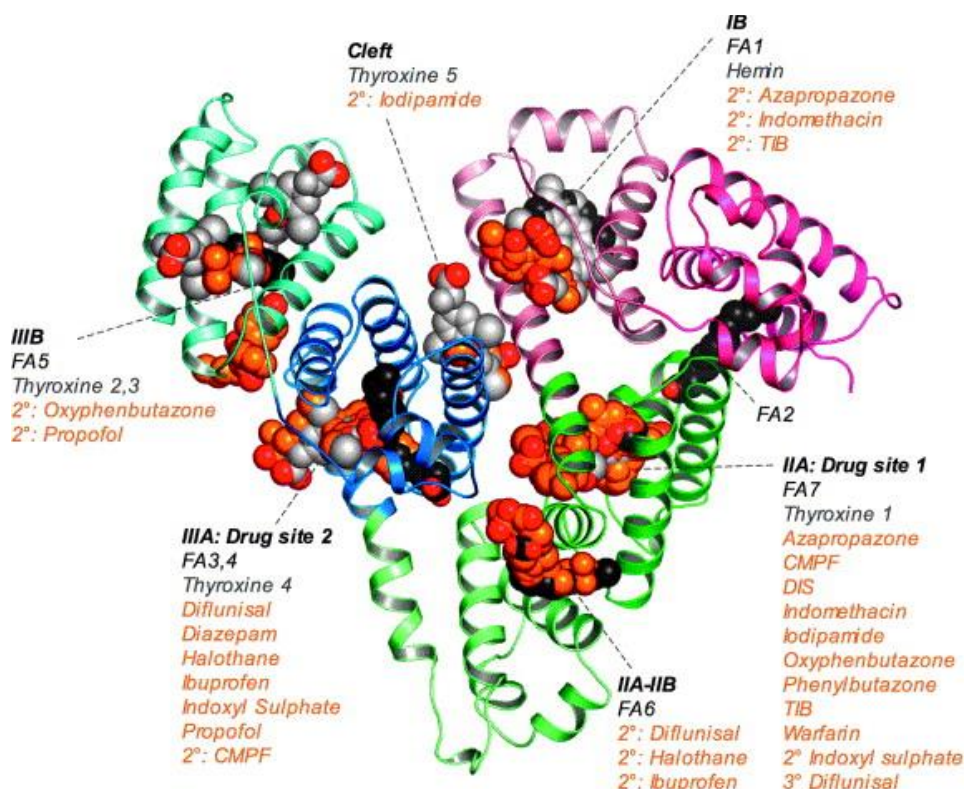


Figure 2.2 Summary of the ligand binding capacity of HSA as defined by crystallographic studies. Ligands are depicted in space-filling representation; oxygen atoms are coloured red; all other atoms in fatty acids (FA), other endogenous ligands (hemin, thyroxine) and drugs are coloured dark-grey, light grey and orange, respectively. Reprinted with permission from Elsevier Ltd: Journal of Molecular Biology (Ghuman, J.; Zunszain, P. A.; Petitpas, I.; Ananyo A. Bhattacharya, A. A.; Otagiri, M.; Curry, S., *J. Mol. Biol.* **2005**, 353 (1), 38-53). Copyright (2005) Elsevier Ltd.

The synthetic goal of this chapter was therefore to decrease plasma protein binding of tetramate analogues by increasing their polarity. The increase in compound polarity was achieved by incorporation of heterocyclic rings at C-2 and C-9 amide pendant positions of the tetramate core (Figure 2.3), which was also correlated with a decrease in lipophilicity and an increase in aqueous solubility. In particular, C-2 *t*-butyl or phenyl groups present in previously synthesised tetramate analogues were replaced with pyridine rings (R^1). C-9 amide pendants (R^2) were diversified into 4-chloro-2-methyl-benzyl, cyclohexyl and polar tetrahydropyranyl and adamantyl groups.

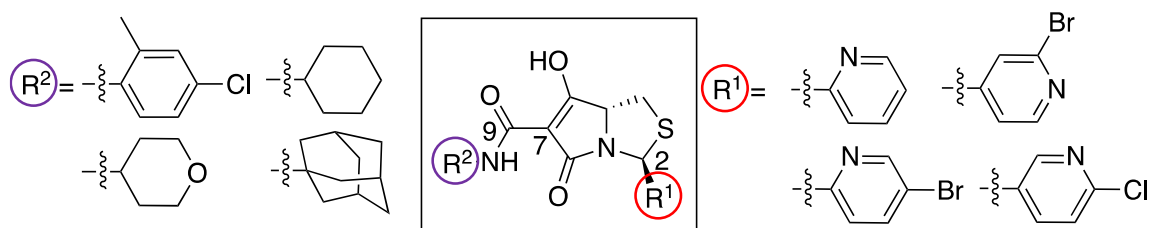


Figure 2.3. Target library of cysteine-derived tetramate analogues.

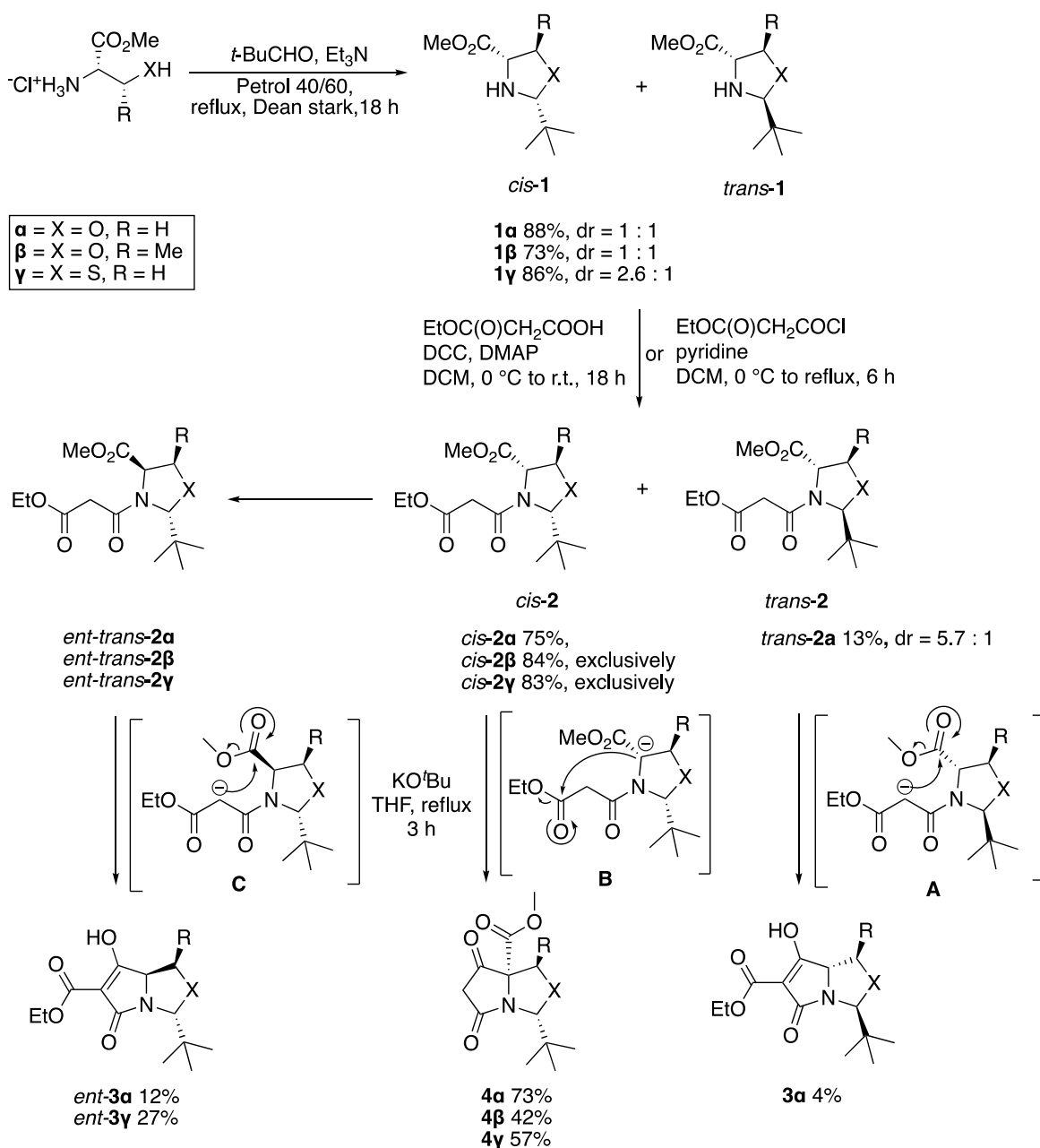
2.2 Reported synthesis of tetramate derivatives: previous work in the Moloney group

The Moloney group has explored and devised a synthetic pathway leading to highly functionalised bicyclic tetramic acid derivatives with high enantiopurity, starting from L-serine, L-threonine and L-cysteine (Scheme 2.1).¹⁰²⁻¹⁰⁹ According to the method developed by Seebach and co-workers,^{100,101} methyl esters hydrochloride salts of respective amino acids were condensed with pivalaldehyde (*t*-BuCHO) in presence of triethylamine (Et_3N) by refluxing in petrol 40/60 with continual removal of water by a Dean-Stark apparatus to yield oxazolidines or thiazolidine **1 α - γ** as a mixture of *cis/trans*-2,5 diastereomers with a diastereomeric ratio (dr) ranging from 1 : 1 to 2.6 : 1.^{102,107,108}

The oxa/thiazolidines were then *N*-acylated either under DCC-DMAP coupling conditions or with the corresponding acid chloride and pyridine as a catalyst to produce malonamides **2 α - γ** in high yield (83 - 88%).^{102,107,108} Interestingly, the malonamides were obtained either predominately (**2 α** , R = H, X = O, *cis/trans* = 5.7 : 1) or exclusively as the *cis* diastereomer, in which the relative stereochemistry was confirmed by NOE analysis and X-ray crystallography.^{102,107,108} Presumably ring-chain tautomerism of the starting oxa/thiazolidines allowed equilibration to the more stable *cis*-malonamides in which both C-2 and C-5 substituents are pseudoequatorial.^{102,105}

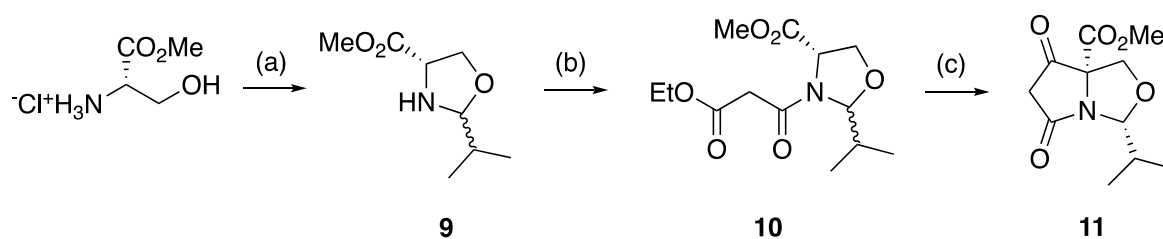
Dieckmann cyclisation of the malonamides **2 α - γ** under the basic conditions of KO^tBu gave bicyclic tetramates **4 α - γ** as the major products.^{102,107,108} The main guiding principle to control the direction of cyclisation was to place the bulky C-2 *t*-butyl on the less hindered *exo*-face of the bicyclic system.¹⁰² Although there was some precedent in which the synthesis of highly functionalised bicyclic lactam systems with *endo*-substituents was reported by Nagasaka and Imai, they were found to be much less stable than the corresponding *exo*-substituents.^{124,125} Consistent with this observation, the major products **4 α - γ** were formed via ring closure from the C-5 enolate onto the ethyl ester, which placed the *t*-butyl group on the *exo*-face of the bicyclic ring system with a *cis* relationship between the C-2 and C-5 substituents (enolate type **B**, Scheme 2.1).^{102,107,108} This route of cyclisation was favoured albeit the reaction was via formation of a relatively unstable enolate. Enantiomeric excess (ee) values of > 90% were reported for the major product **4 α** .¹⁰² In the case where minor *trans* diastereomer of the malonamide was present, the malonamide *trans*-**2 α** cyclised from the more stable side chain enolate onto the C-5 methyl ester to yield a minor tetramate ester product **3 α** in 4% yield, again

placing the C-2 *t*-butyl group on the less hindered *exo*-face of the bicyclic system (enolate type **A**, Scheme 2.1).¹⁰² Moreover, some epimerisation at C-5 of *cis*-**2** could occur under the basic conditions, leading to *ent*-*trans*-**2**. Similarly, this malonamide *ent*-*trans*-**2** could also cyclise by closure of the side chain enolate onto the methyl ester at C-5 forming the minor product *ent*-**3** (via enolate type **C**, Scheme 2.1).^{102,107,108}



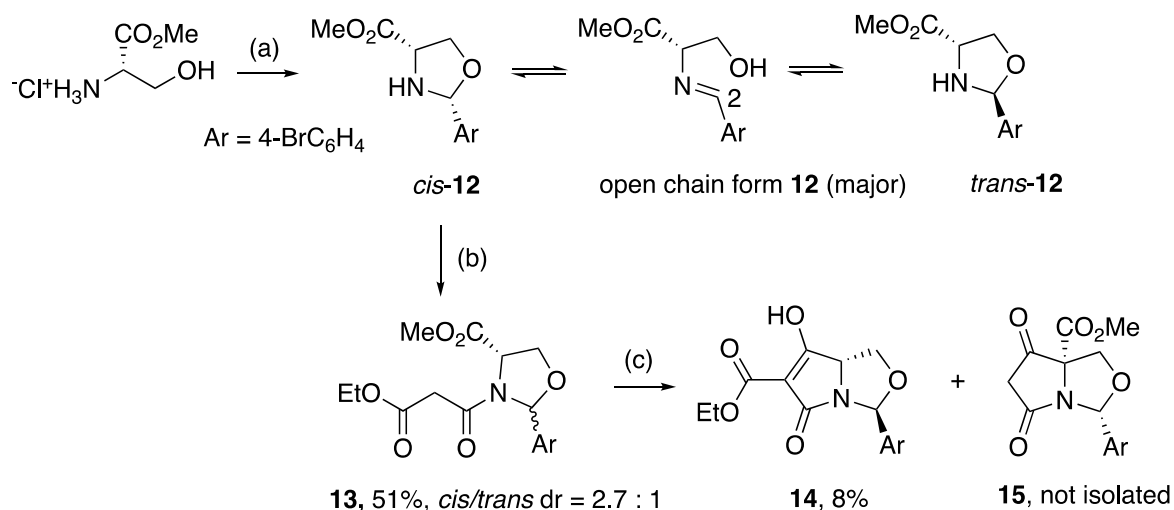
Scheme 2.1 Synthesis of tetramate derivatives with C-2 *t*-butyl group.^{102,107,108}

With the original condensing species limited to pivalaldehyde, the synthetic approach led to tetramic acid derivatives with high hydrophobicity. Attempts to expand the synthetic scope at C-2 were carried out by replacement of pivalaldehyde with isobutyraldehyde (*i*PrCHO) and substituted benzaldehydes in both L-serine derived oxazolidine systems and L-cysteine derived thiazolidine systems. Unfortunately, difficulties were encountered with these oxazolidine systems. The synthesis of oxazolidine derived tetramate **11** with isobutyraldehyde as the initial condensing species was not fruitful (Scheme 2.2).¹²⁶ Although LRMS and ¹H NMR analysis both confirmed the presence of the product **11** and all intermediates (**9** and **10**) in the synthetic pathway, no compound could be isolated from flash silica column chromatography.¹²⁶ Moreover, the ¹H NMR spectrum for the crude product of **11** only showed a few detectable peaks with other unpredictable signals, suggesting that the Dieckmann cyclisation was not as straightforward as that with C-2 *t*-butyl group and the yield for **11** was low.¹²⁶



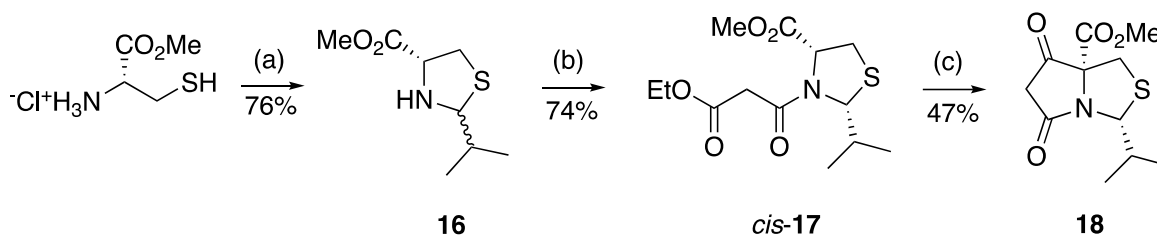
Scheme 2.2 Attempted synthesis of oxazolidine derived tetramate with C-2 isopropyl group.¹²⁶ *Reagents and conditions:* (a) CH(CH₃)₂CHO, Et₃N, petrol 40/60, reflux with Dean-Stark apparatus, 18 h; (b) mono-ethyl malonate, DCC, DMAP, DCM, 0 °C to r.t., 6 h; (c) KO^tBu, THF, reflux, 3 h.

Similarly, synthesis leading to oxazolidine-derived tetramates with substituted benzaldehydes as initial condensing species suffered from instability of the resulting systems.^{109,127} Condensation according to a previously reported procedure in methanol at room temperature for 2 hours successfully yielded oxazolidine **12** in excellent yield (98%, Scheme 2.3).^{127,128} However, the ¹H NMR spectra for oxazolidine **12** suggested the presence of a predominant open-chain form, based on the azomethine proton (H-2) chemical shift, in addition to *cis* and *trans* ring forms of the oxazolidine.¹²⁷ This instability resulting from replacement of the bulky C-2 *t*-butyl group with planar aromatic systems could be partially circumvented by *N*-acylation, which 'locked' the oxazolidine in its ring form, driving the ring-chain tautomeric equilibrium in its favour. The *N*-acylated oxazolidine **13** was produced in moderate yield of 51% with a *cis/trans* ratio of 2.7 : 1 (Scheme 2.3).¹²⁷ As compared to the predominant (5.7 : 1) or exclusive formation of *cis*-malonamides in the C-2 *t*-butyl series, the stereochemical control exerted by the planar aromatic C-2 substituent was less strong. The malonamide **13** was cyclised as a mixture of diastereomers under basic conditions of Dieckmann cyclisation. Only tetramate ester **14** was isolated from flash column chromatography in 8% yield but it was unstable to acidic wash (Scheme 2.3).¹²⁷ Although tetramate **15** was detected by mass spectrometry, it was not isolated (Scheme 2.3).¹²⁷ Given the low yield of the reaction and instability of the resulting tetramate system, the synthesis of oxazolidine derived tetramates with C-2 variations was not carried forward.



Scheme 2.3 Attempted synthesis of oxazolidine derived tetramate with C-2 bromobenzene group.¹²⁷ *Reagents and conditions:* (a) 4-BrC₆H₄CHO, Et₃N, methanol, r.t., 2 h; (b) mono-ethyl malonate, DCC, DMAP, DCM, 0 °C to r.t., 3-5 h; (c) KO^tBu, THF, reflux, 3 h.

In contrast to the oxazolidine derived systems, thiazolidine systems derived from L-cysteine allowed greater variations at C-2 without compromising their stability.^{109,126} With the increased bulkiness of S over O, thiazolidines are known for their preference to exist in the ring-closed form than the open-chain form.¹²⁹ Condensing L-cysteine methyl ester hydrochloride with isobutyraldehyde produced thiazolidine **16** in good yield (76%) as a 2 : 1 mixture of *cis/trans* diastereomers (Scheme 2.4).¹²⁶ The subsequent synthetic route followed that of the C-2 *t*-butyl series, in which *cis*-malonamide **17** was predominantly formed (*trans*-**17** detected but not isolated) and further cyclised to produce bicyclic tetramate **18** with a *cis* relationship between C-2 and C-5 substituents (Scheme 2.4).¹²⁶

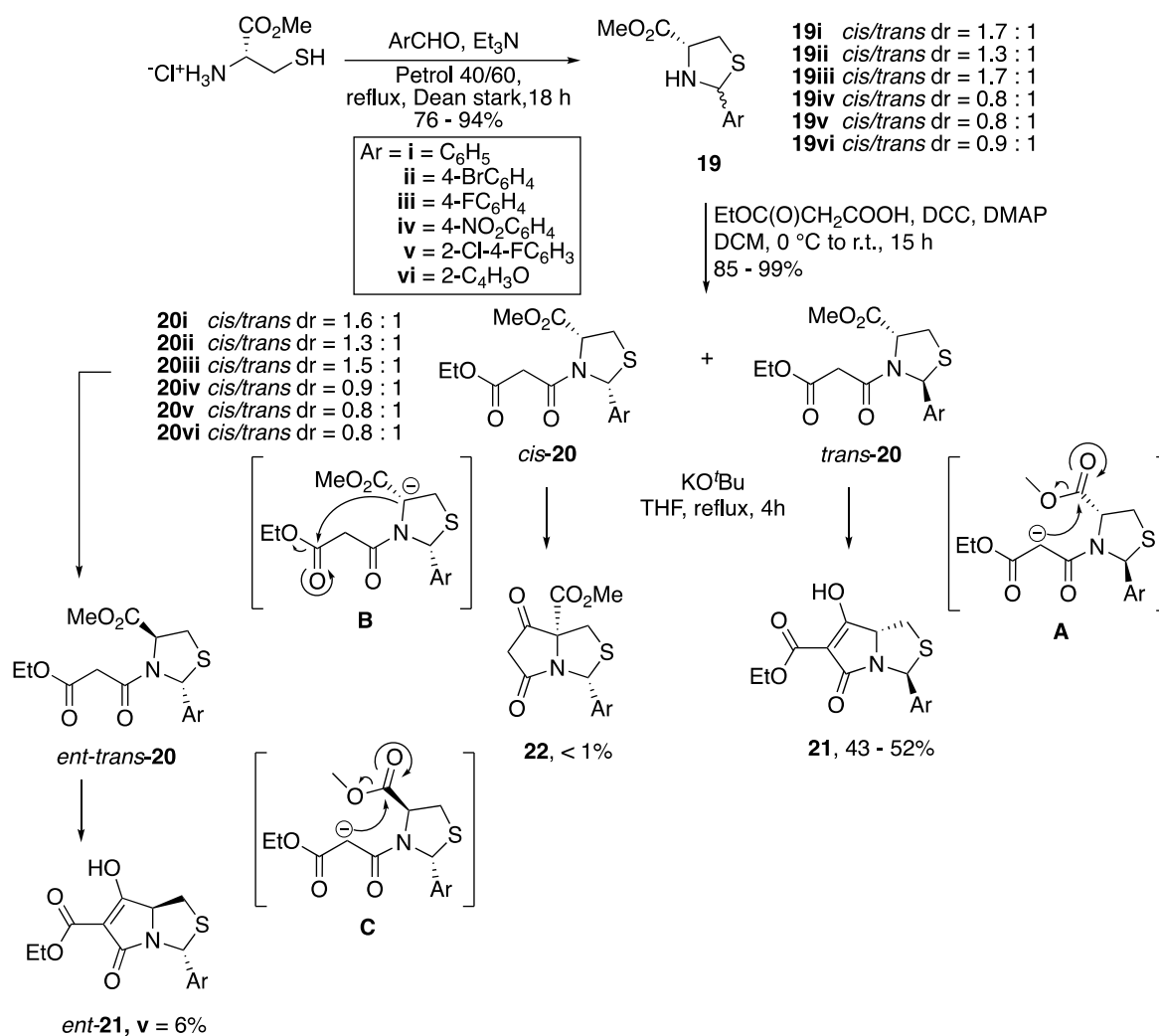


Scheme 2.4 Synthesis of thiazolidine derived tetramate with C-2 isopropyl group.¹²⁶

Reagents and conditions: (a) $\text{CH}(\text{CH}_3)_2\text{CHO}$, Et_3N , petrol 40/60, reflux with Dean-Stark apparatus, 16 h; (b) mono-ethyl malonate, DCC, DMAP, DCM, 0 °C to r.t., 6 h; (c) KO^tBu , THF, reflux, 3 h.

Condensation of L-cysteine methyl ester hydrochloride with substituted benzaldehydes and furfural gave stable thiazolidines **19i-vi** in good yield (76 - 94%), which were then converted to the corresponding malonamides **20i-vi** (85 - 99% yield), with the *cis/trans* diastereomeric ratio (ranging from 1.6 : 1 to approximately 1 : 1) preserved from the starting thiazolidines in all cases (Scheme 2.5).¹⁰⁹ This observation was contrasted with all the above-mentioned systems where the free *cis-trans* equilibrium led to a preferred formation of *cis*-malonamides. Malonamides **20i-vi** were cyclised as a mixture of diastereomers with KO^tBu to form bicyclic tetramate esters **21i-vi** in good yield (43 - 52%) with a *trans* relationship between H-2 and H-5 (Scheme 2.5).¹⁰⁹ The planar aromatic C-2 substituents exerted a less strong steric influence in ring closure than C-2 *t*-butyl and isopropyl groups, leading to stark changes in the selectivity of the process.¹⁰⁹ In addition to the preference of C-2 substituents to be placed on the *exo*-bicyclic face, the route of cyclisation was strongly influenced by formation of the more stable side chain enolate intermediates.¹⁰⁹ Side chain enolates type **A** formed from *trans*-**20** malonamides readily fulfilled these two requirements and were thus preferentially cyclised to give the major

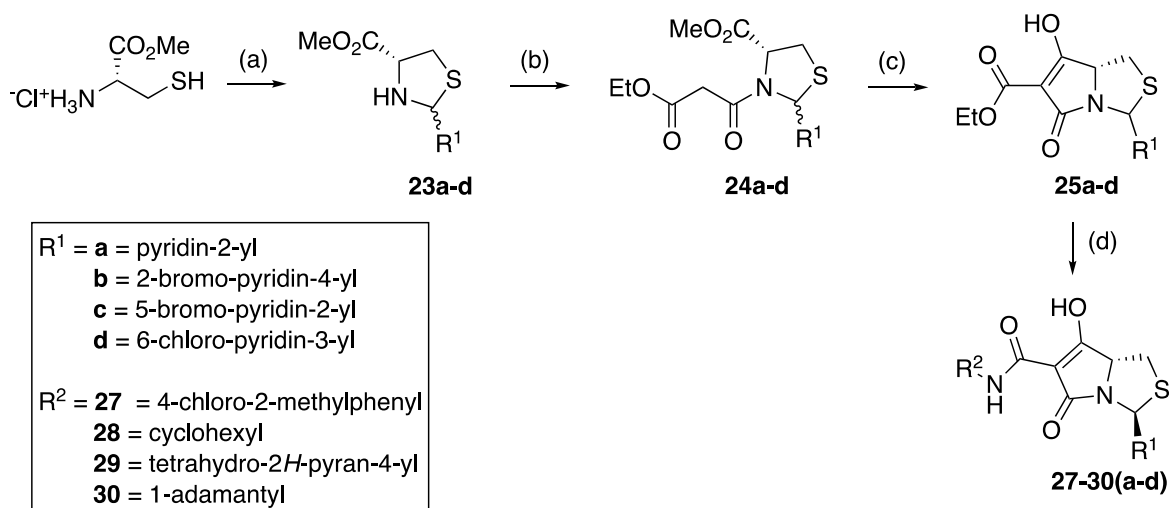
products **21** (Scheme 2.5). Placing the C-2 groups on the *exo*-face in *cis*-**20** led to ring closure from the less stable C-5 enolate type **B** onto the side chain ethyl ester, giving C-5 methyl ester tetramates **22**, which were detected by mass spectrometry but only isolated in some cases with < 1% yield (Scheme 2.5).¹⁰⁹ Some epimerisation at C-5 for *cis*-**20** could occur under the basic conditions to produce *ent-trans*-**20**, which then cyclised from the side chain enolate onto the C-5 methyl ester (enolates type **C**), giving *ent*-**21** as a minor product (*ent*-**21v**, Ar = 2-Cl-4-FC₆H₃, 6% yield, Scheme 2.5).¹⁰⁹ Given that cyclisation and epimerisation occurred simultaneously, the presence of *ent*-**21** explained the erosion of ee of the products from Dieckmann cyclisation. Reported ee values of the tetramate esters **21i-vi** were in the range of 76% - 83% as compared to > 90% ee in the corresponding tetramate systems **4**.^{102,108,109}



Scheme 2.5 Synthesis of thiazolidine-derived tetramates with C-2 aromatic rings.¹⁰⁹

2.3 Synthetic route leading to cysteine-derived tetramates with C-2 heterocycles

It was evident based on the previous work in the Moloney group that cysteine-derived tetramate derivatives were attractive templates to further expand the diversity at C-2, both because of their better stability over their oxazolidine derived counterparts and the change in chemoselectivity to favour the formation of tetramate esters, allowing for late-stage functionalisation to tetramate carboxamides by transamidation reactions.^{48,109,110} This synthetic pathway thus allowed incorporation of heterocycles at both C-2 and C-9, generating the target cysteine-derived tetramate library to increase molecular polarity.

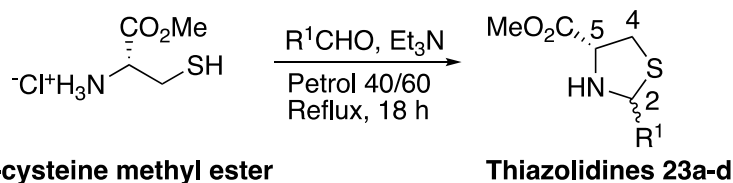


Scheme 2.6 Synthesis of targeted tetramate analogues from L-cysteine methyl ester HCl.

Reagents and conditions: (a) $R^1\text{CHO}$, Et_3N , petrol 40/60, reflux with Dean-stark, 18 h; (b) mono-ethyl malonate, DCC, DMAP, DCM, 0 °C to r.t., 18 h; (c) KO^tBu , THF, reflux, 4 h; (d) $R^2\text{NH}_2$, THF/toluene (1 : 4), reflux, 18 h.

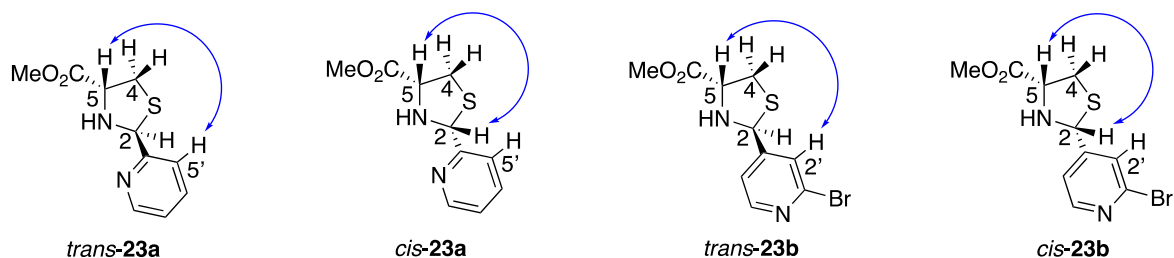
2.3.1 Condensation with respective pyridyl aldehydes

Condensation of L-cysteine methyl ester hydrochloride with four different pyridine-carboxaldehydes gave the corresponding stable thiazolidines **23a-d** in good yield (75 - 86%) as a mixture of *cis*- and *trans*-diastereomers of approximately equal ratio (Table 2.1). The relative stereochemistry was distinguished by a difference in the chemical shift of H-2, with the value of the *trans*-isomer consistently being more downfield than that of the *cis*-isomer (Table 2.1). This trend in the H-2 chemical shifts of *cis* and *trans* thiazolidines was consistent with that previously found in thiazolidines with C-2 substituted aromatic rings.¹⁰⁹ The trend is preserved in H-5 chemical shifts. The assignment of stereochemistry was further confirmed by NOE analysis, where the presence of an enhancement between H-5 proton and an aromatic proton suggested *trans*-stereochemistry and an enhancement between H-5 and H-2 protons suggested *cis*-stereochemistry (Figure 2.4).

Table 2.1 Yield and H-2, H-5 chemical shifts (CDCl₃, 400 MHz) for thiazolidines.

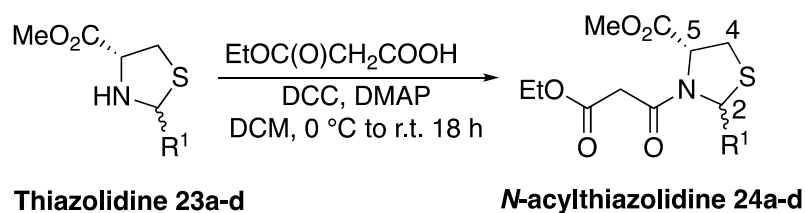
R ¹ =	δ_H (ppm) for H-2		δ_H (ppm) for H-5		<i>cis/trans</i>	% Yield ^a
	<i>cis</i>	<i>trans</i>	<i>cis</i>	<i>trans</i>		
23a 	5.65	5.84	4.03 (dd)	4.55 (dd)	1.2 : 1	75
23b 	5.47	5.75	3.99 (q)		0.6 : 1	86
23c 	5.60	5.80	4.01 (dd)	4.47 (dd)	1.0 : 1	80
23d 	5.48	5.77	3.96 (app t)	4.02 (app t)	0.8 : 1	83

[a] Total isolated yield was calculated based on a mixture of *cis* and *trans* diastereomers.

**Figure 2.4** NOE analysis of thiazolidines **23a** and **23b**.

2.3.2 N-Acylation

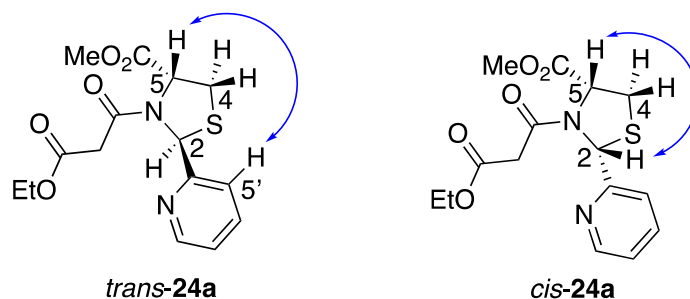
The thiazolidines **23a-d** were converted by DCC coupling with mono-ethyl malonate to the corresponding *N*-acylthiazolidines **24a-d** in high yield (77 - 92%) with a *cis/trans* ratio indicating a stronger preference towards the formation of a *cis*-isomer (Table 2.2). The *cis/trans* ratios were similar to those previously reported in thiazolidine-derived systems with C-2 aromatic rings, ranging from 1.6 : 1 to 0.8 : 1 (Scheme 2.5, page 40, *vide supra*).

Table 2.2 Yield and H-2 chemical shift data (CDCl₃) for *N*-acylthiazolidines.

R ¹ =	δ_{H} (ppm) for H-2				<i>cis</i> / <i>trans</i>	% Yield ^a
	<i>cis</i>		<i>trans</i>			
	Major rotamer	Minor rotamer	Major rotamer	Minor rotamer		
24a 	6.18	6.45	6.16	6.25	1.2 : 1	84
24b 	6.14	6.22	6.12	6.12	1.6 : 1	92
24c 	6.19	6.21	6.15	6.18	1.3 : 1	85
24d 	6.23	Obscured by <i>cis</i> major	6.21	6.17	1.2 : 1	77

[a] Total isolated yield was calculated based on a mixture of *cis* and *trans* diastereomers.

Similar to thiazolidines **23a-d**, characteristic H-2 chemical shifts of the acylated products **24a-d** were observed, with each diastereomer appearing as a rotameric pair. For **24a**, the *cis*- and *trans*-diastereomers were separated by flash column chromatography, where the assignment of relative stereochemistry was supported by NOE analysis (Figure 2.5).

**Figure 2.5** NOE analysis of *N*-acylthiazolidines **24a**.

Although 2D NOESY experiments were not sensitive enough to detect weak nOe effects in this case (the aforementioned NOE analysis to confirm stereochemistry used 1D nOe experiments), they nonetheless confirmed the presence of rotamers in *N*-acylthiazolidines. Rotamers occurred because of the restricted amide bond rotation (Figure 2.6). Between the pair of rotamers from the same diastereomer, there were consistent and dynamic proton exchanges, which can be distinguished from NOE signals in the 2D NOESY spectrum (Figure 2.7). In 2D NOESY spectra of small molecules (MW \leq 500) in low viscous solvent (e.g. CDCl₃), peaks arising from chemical exchange processes have the same sign as the diagonal (red) while NOE cross peaks (blue) are of opposite sign.¹³⁰ Cross peaks δ (6.48, 6.20) or δ (6.20, 6.48) of H-2 chemical shifts, which were of the same sign as the diagonal peaks, indicated that they occurred due to a dynamic chemical exchange and hence supported that these chemical shifts belonged to a pair of rotamers arising from the same diastereomer.

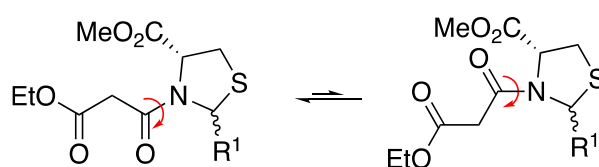


Figure 2.6 Rotameric behaviour of *N*-acylthiazolidines from amide bond rotation.

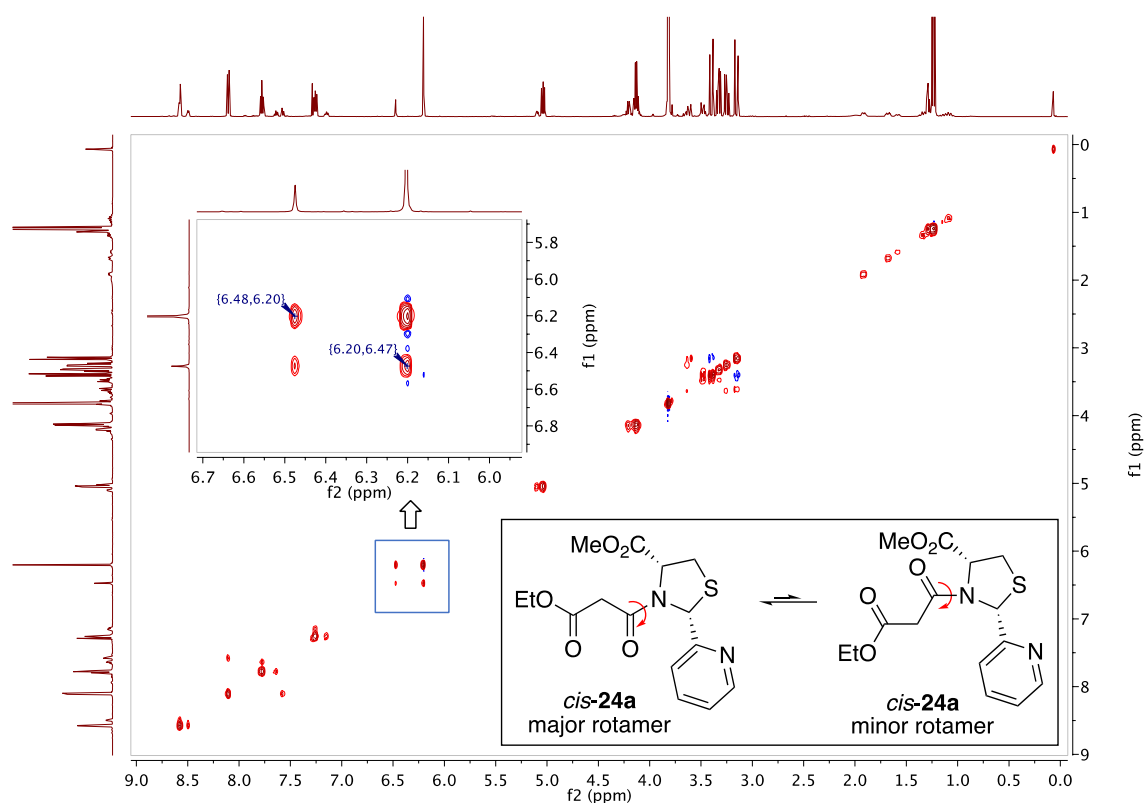


Figure 2.7 2D NOESY spectrum of *cis*-**24a** confirming the presence of rotamers. 2D NOESY and EXSY (exchange spectroscopy) are essentially the same NMR experiment, which is used as a relatively easy technique with readily set-up automation to study rotamers instead of variable temperature (VT) experiments.¹³⁰

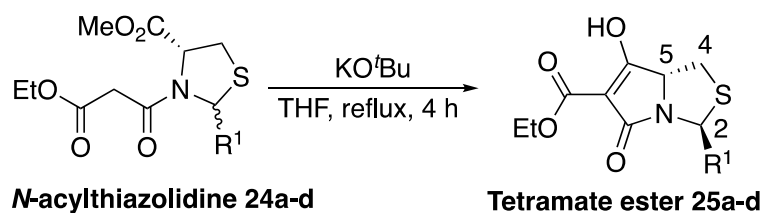
By tabulation of the H-2 chemical shifts of *N*-acylthiazolidines **24a-d**, it was observed that for the major rotamers, H-2 chemical shift of the *trans*-isomer was invariably more upfield than that of the *cis*-isomer, which was opposite to that observed in the thiazolidine series (Table 2.2, page 43; Table 2.1 page 42, *vide supra*). This trend seemed to be conserved for minor rotamers. Interestingly, H-2 chemical shifts of C-2 furfural malonamide **20vi** also demonstrated this trend, which was taken as an abnormality in the previously reported series with C-2 aromatic rings (Scheme 2.5, page 40, *vide supra*).¹⁰⁹

The change in the *cis/trans* ratio from the starting thiazolidines **23a-d** to *N*-acylthiazolidines **24a-d** (Table 2.2, page 43; Table 2.1 page 42, *vide supra*) suggested an interconversion between the two diastereomers during the course of acylation, with the equilibrium leading to a preferred formation of the *cis*-malonylthiazolidines; this type of behaviour has been observed previously in C-2 *t*-butyl and isopropyl series.^{102,107,108,126} This observation was contrasted with the previously reported conservation of diastereomeric ratios through the course of acylation in thiazolidines with C-2 aromatic rings (Scheme 2.5, page 40, *vide supra*).¹⁰⁹

2.3.3 Dieckmann cyclisation

Dieckmann cyclisation of *N*-acylthiazolidines **24a-d** with KO^tBu successfully gave stable bicyclic tetramate esters **25a-d** in 35 - 48% yield with complete enolisation as observed by ¹H NMR spectra and with characteristic H-2 and H-5 chemical shift values for the bicyclic ring system consistent to those previously reported in thiazolidine-derived tetramate esters with C-2 substituted aromatic rings (Table 2.3).¹⁰⁹ Each tetramate ester appeared as a single diastereomer with a *trans* relationship between H-2 and H-5 across the bicyclic ring systems. The relative stereochemistry of **25a** was confirmed by NOE analysis (Figure 2.8). On the basis of the consistent and characteristic H-2 chemical shifts, the same stereochemistry was assigned for other tetramate esters in the series.

Table 2.3 Yield and H-2, H-5 chemical shifts (methanol-*d*₄, 400 MHz) for tetramate esters.



	R ¹ =	δ_{H} (ppm) for H-2	δ_{H} (ppm) for H-5	% Yield
25a		6.36	4.40 (app t)	48
25b		6.30	4.17 – 4.24 (m)	35
25c		6.25	4.79 – 4.87 (obscured) ^a	40
25d		6.28	4.83 (app t)	41

[a] The H-5 chemical shift in **25c** was obscured by water in methanol-*d*₄ and thus subsequent characterisation for this compound was carried in CDCl₃ (H₅, 4.88, t).

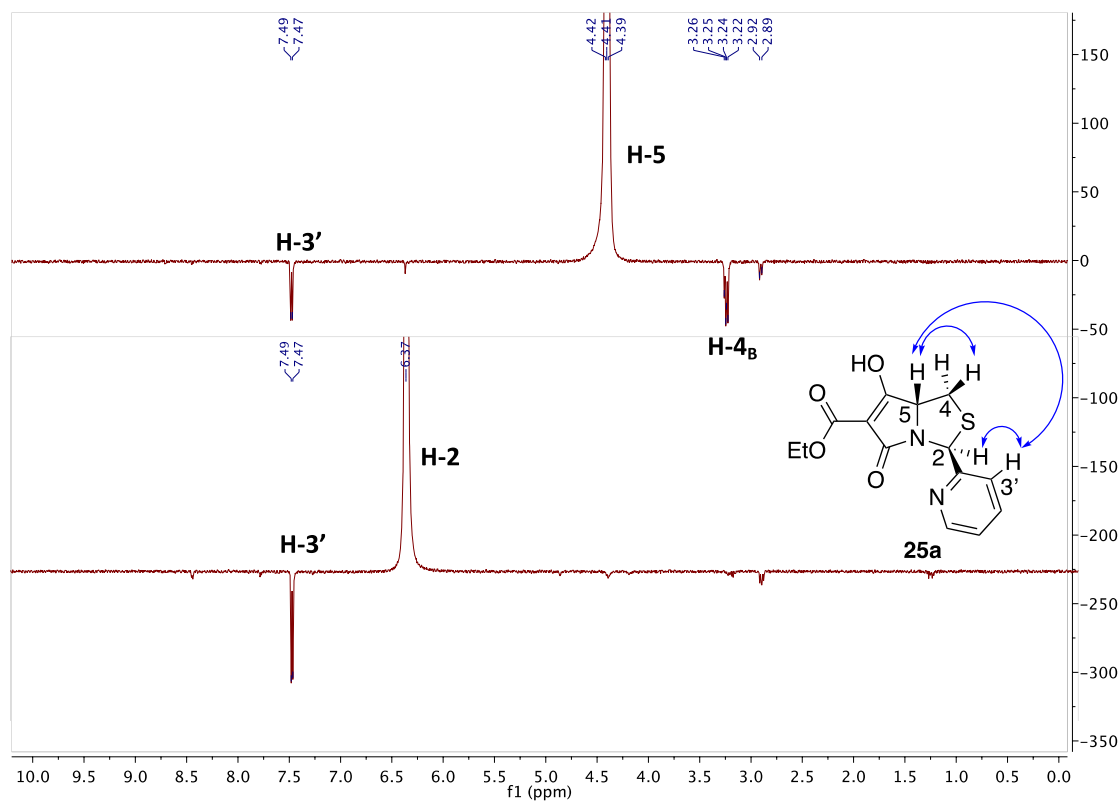


Figure 2.8 NOE analysis of tetramate ester **25a**.

The predominant pathway of cyclisation starting from the *trans*-malonylthiazolidines, *trans*-**24a-d** to **25a-d** suggested that the reaction proceeded preferentially by closure of the more stabilised malonamide side chain enolate onto the C-5 ester (type **A**), which also placed the C-2 heterocycles on the less hindered *exo*-face (Scheme 2.7). This preferred route of ring closure was consistent with that previously observed in thiazolidine derived systems with C-2 aromatic rings.¹⁰⁹

The alternatively cyclised product **26** was detected by mass spectrometry and was isolated from the reaction of **24d** in 3% yield. The NOE analysis of **26d** suggested a relative *cis*-stereochemistry between C-2 substituent and C-5 methyl ester (Figure 2.9), indicating that the alternative mode of cyclisation, starting from *cis*-**24d** proceeded by closure of the less stable C-5 enolate onto the ethyl ester (type **B**, Scheme 2.7), which preferentially placed the C-2 group on the *exo*-face. This minor route of cyclisation was also reported previously in thiazolidine derived systems with C-2 aromatic rings in < 1% yield.¹⁰⁹

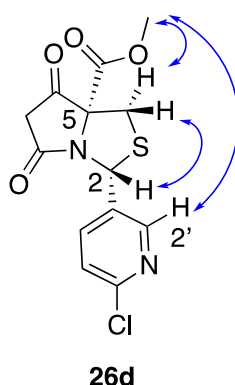
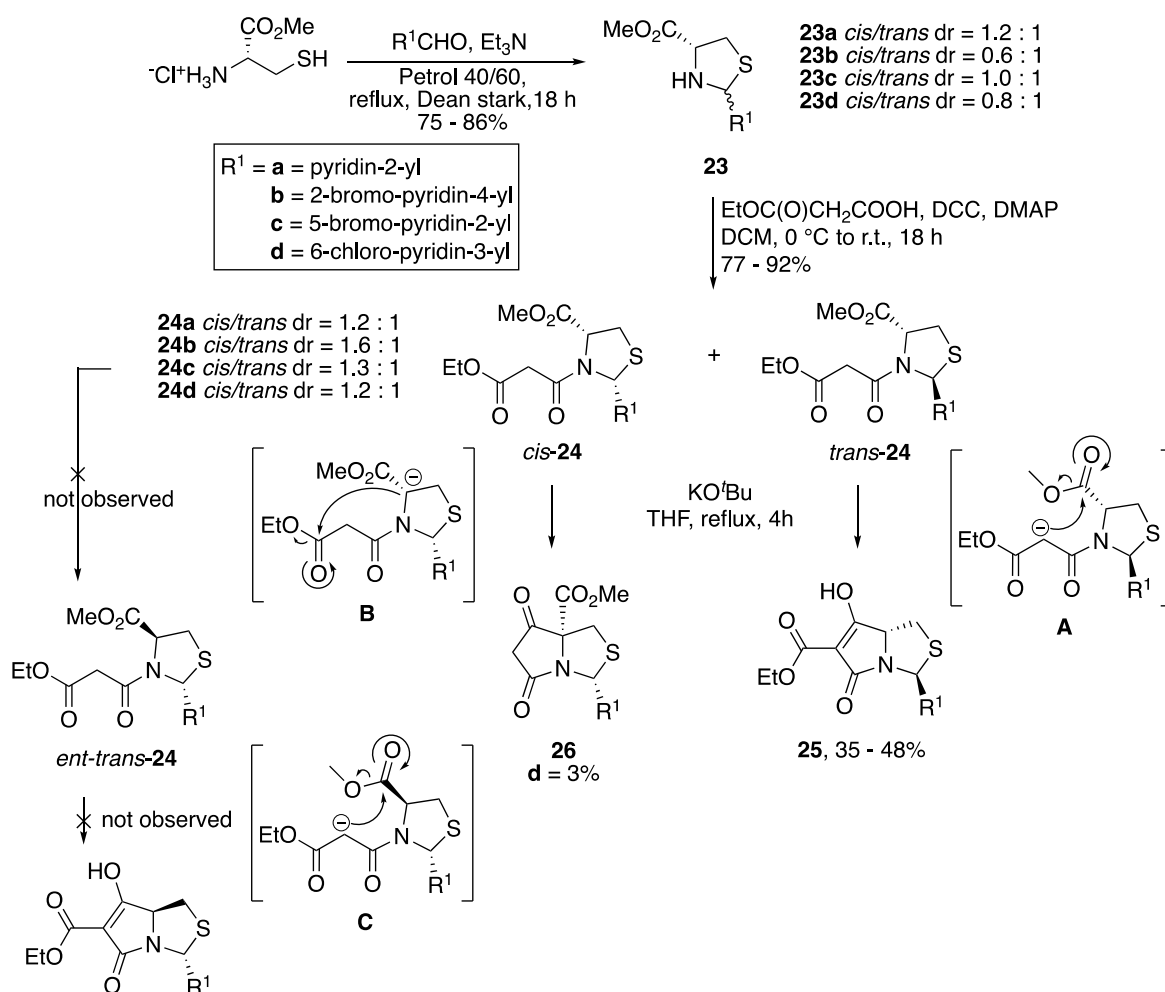


Figure 2.9 NOE analysis of alternatively cyclised tetramate product **26d**.

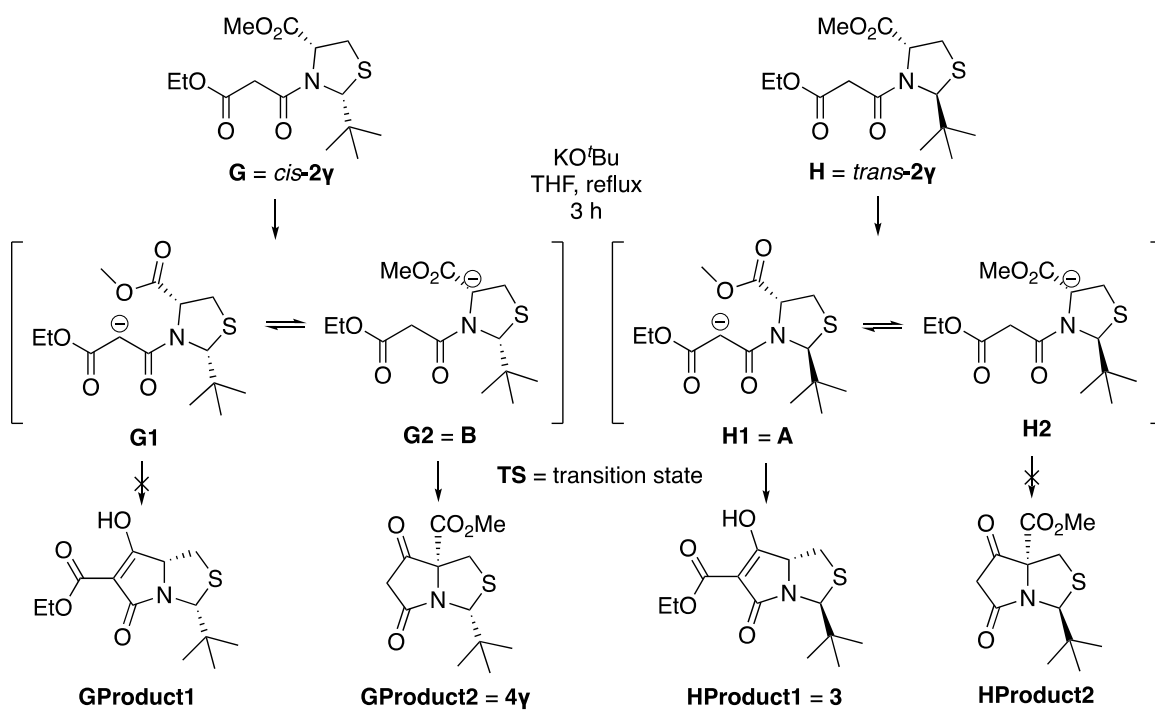
It was also suggested that Dieckmann cyclisation could occur after epimerisation at C-5 of *cis*-**24** under the basic conditions of the reaction giving *ent-trans*-**24**.¹⁰⁹ While the *cis*- and *trans*-diastereomers of malonamides **24b-d** could not be easily separated and the mixture was used for the subsequent Dieckmann cyclisation, the simultaneous epimerisation and cyclisation gave concerns about erosion of enantiopurity for the products.¹⁰⁹ In the case of **24a**, the two diastereomers were able to be separated by flash column chromatography and each was taken for separate cyclisation. Reaction of malonamide *trans*-**24a** with KO^tBu successfully gave the cyclised product **25a**. However, the process of cyclisation after potential epimerisation of *cis*-**24a** was not observed (cyclisation via type C enolate, Scheme 2.7) and the cyclisation of *cis*-**24a** only gave degraded product. This could be attributed to intrinsically low yield of the process as well as to the extended time of reaction under reflux, leading to degradation of the starting malonamide.



Scheme 2.7 Synthesis of thiazolidine-derived tetramates with C-2 pyridine rings.

In view of the stark contrast in the chemoselectivity of Dieckmann cyclisation with a point change at C-2 from bulky *t*-butyl group or isopropyl group to planar aromatic systems (substituted phenyl rings or heterocycles), density-functional theory (DFT) calculations have been carried out by Dr Xiang Li to model the energy profile of the cyclisation process in thiazolidine systems with C-2 *t*-butyl group (Scheme 2.8, Figure 2.10) and phenyl ring (Scheme 2.9, Figure 2.11). To simplify the model, the calculations did not take into account of potential epimerisations at C-5 under the basic conditions of the process.

In the cyclisation of *N*-acylthiazolidines **2 γ** with C-2 *t*-butyl group, the major C-5 methyl ester tetramate product **4 γ** (**GProduct2**, 57% yield)¹⁰² formed from *cis*-**2 γ** (**G**) followed the energy pathway with the least activation energy ($E_a = 5.3$ kcal/mol). The alternative pathway from the same starting material **G** to **GProduct1**, by placing the C-2 *t*-butyl group onto the *endo*-face of the bicyclic ring, would result in a much higher activation energy ($E_a = 17.8$ kcal/mol). The significant difference in the activation energy ($\Delta E_a = 12.5$ kcal/mol) strongly favoured the pathway leading to **GProduct2**, which was also the more stable product (minimum energy = -75.4 kcal/mol) as compared to **GProduct1** (minimum energy = -70.8 kcal/mol). The other tetramate product **HProduct1** from *trans*-**2 γ** (**H**) was not observed because the previous step of *N*-acylation resulted in an exclusive formation of *cis*-**2 γ** (**G**).¹⁰² Given that **HProduct1** possessed the lowest energy (minimum energy = -78.8 kcal/mol) and the activation energy ($E_a = 7.7$ kcal/mol) was not significantly higher ($E_a = 7.7$ kcal/mol), **HProduct1** could potentially form if the *trans*-**2 γ** (**H**) was present in the starting material. The corresponding form of **HProduct1** in oxazolidine system with C-2 *t*-butyl group **3 α** was indeed observed with 4% yield.¹⁰² This model suggested that for *cis*-malonamides with C-2 *t*-butyl group, Dieckmann cyclisation was under a strong kinetic control.



Scheme 2.8 Cyclisation pathway of malonamides **2 γ** and legend for **Figure 2.10**.

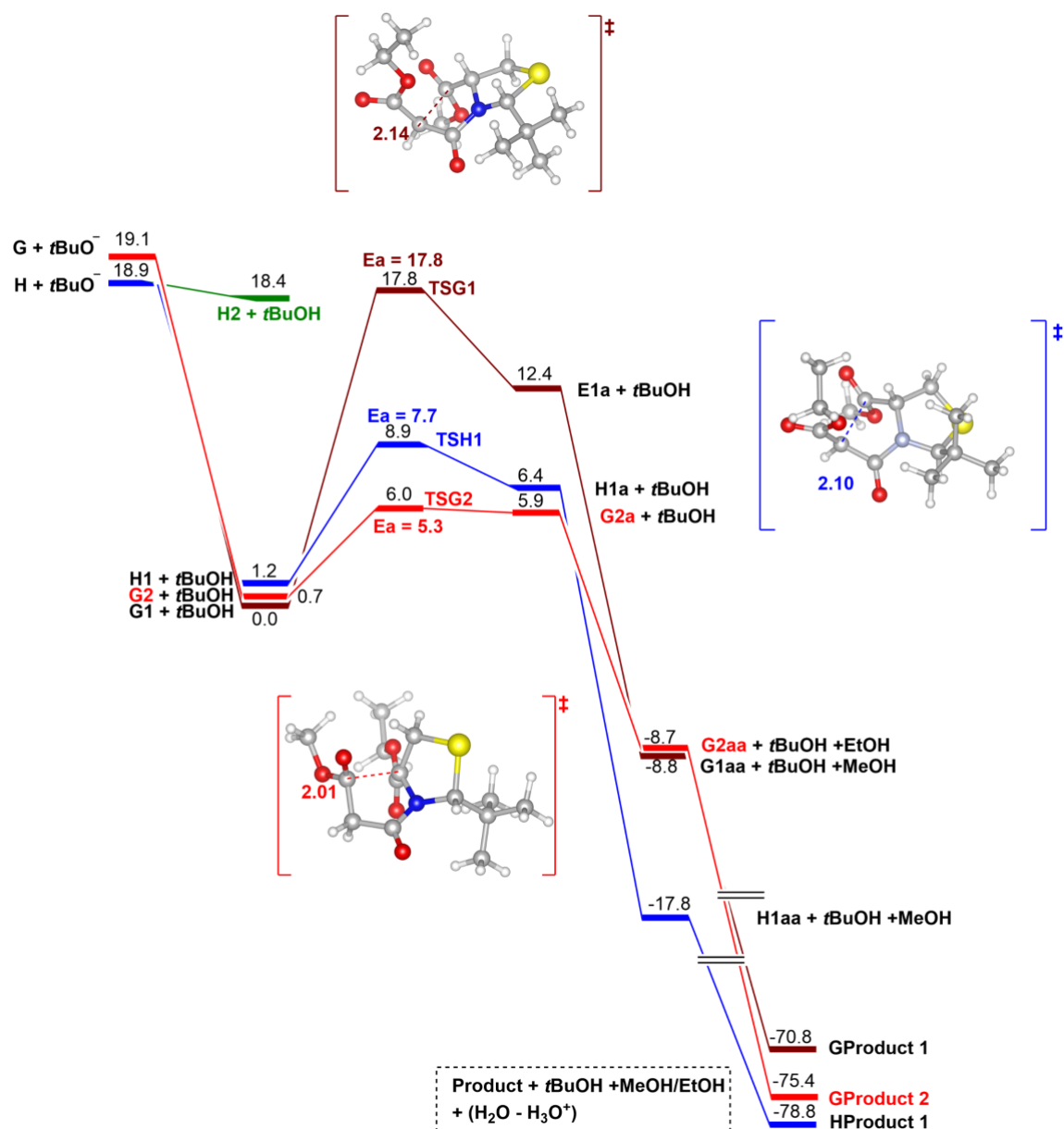
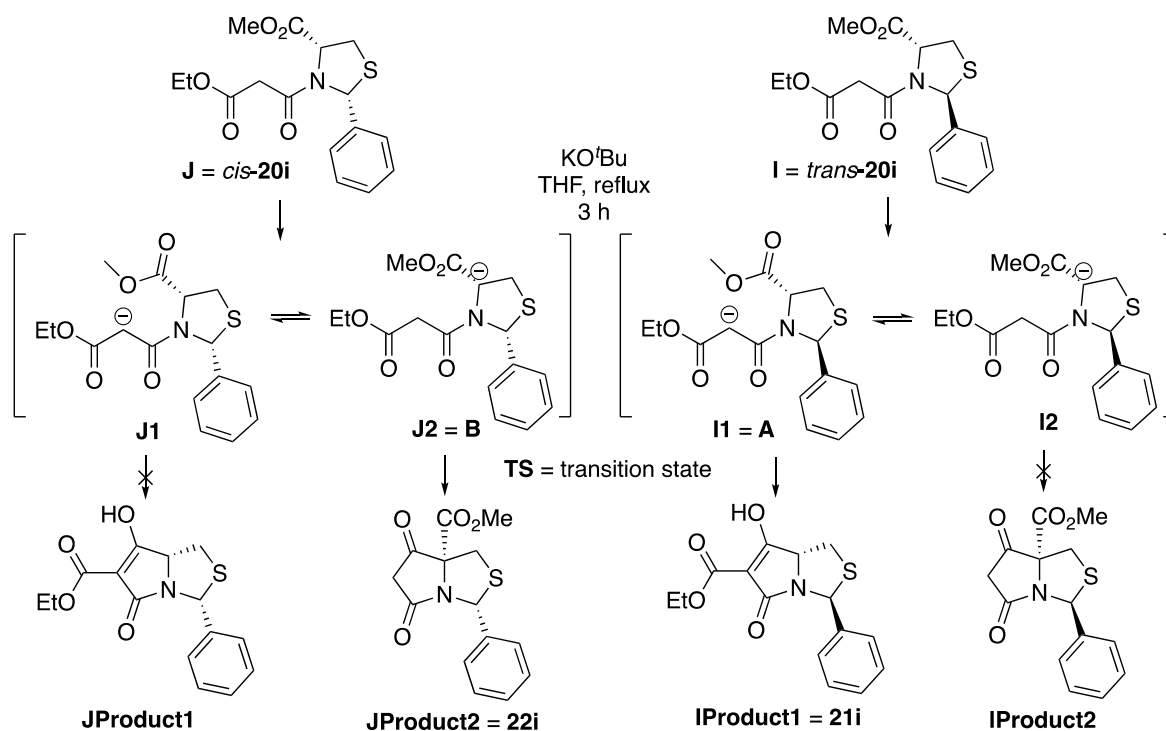


Figure 2.10 Reaction energy profile of Dieckmann cyclisation for malonamides of C-2 *t*-butyl group. Calculated potential energy surfaces at the B3LYP/6-311++G (d,p) level of theory with SMD solvation (THF). All energies are reported in kcal/mol and referenced to the lowest energy carbanion generated after initial deprotonation. Figure and calculations obtained from Dr Xiang Li.

In comparison, tetramate ester **21i** (**IProduct1**) was formed as the major product (43% yield) in the cyclisation of *cis*- and *trans*-malonylthiazolidines **20i** with C-2 phenyl group.¹⁰⁹ The pathway started from *trans*-**20i** (**I**), with the more stable side chain enolate intermediate (**I1**) closing onto the C-5 ester. **IProduct1** appeared to be the thermodynamic product of the process with minimum energy at -75.2 kcal/mol, although the difference in energy to **JProduct1** and **JProduct2** was small. The pathway from *cis*-**20i** (**J**) favoured the formation of **JProduct2** with the least activation energy ($E_a = 7.4$ kcal/mol) of all represented pathways and the smaller minimum energy as compared to **JProduct1**. The reason why **JProduct2** was only observed in < 1% yield from a starting mixture enriched in *cis*-**20i** (**J**) (*cis/trans* = 1.7 : 1)¹⁰⁹ could not be explained by this model. In contrast to the C-2 *t*-butyl series, Dieckmann cyclisation for malonamides with C-2 aromatic rings was no longer under a strong kinetic control; a thermodynamic factor was suggested by this model for the observed chemoselectivity in the reaction.



Scheme 2.9 Cyclisation pathway of malonamides **20i** and legend for **Figure 2.11**.

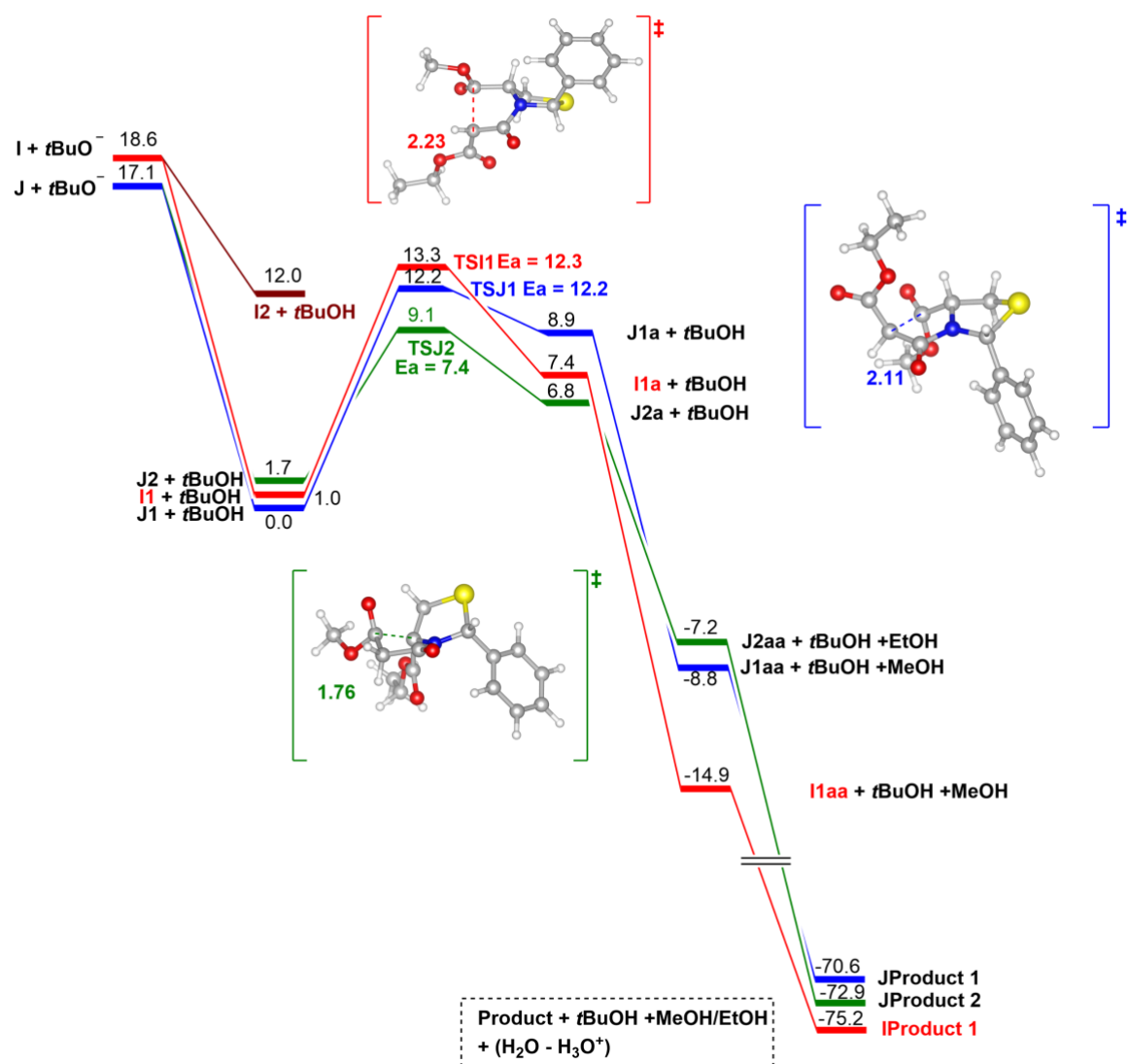
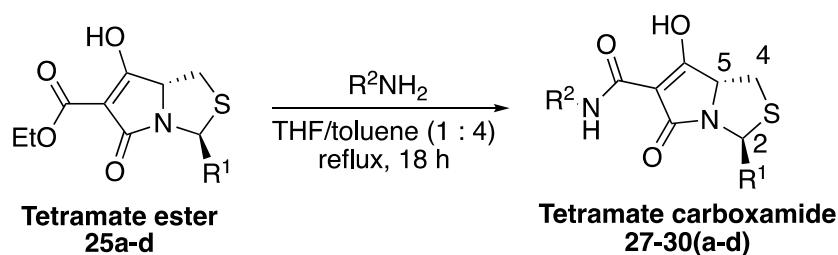


Figure 2.11 Reaction energy profile of Dieckmann cyclisation for malonamides with C-2 phenyl group. Calculated potential energy surfaces at the B3LYP/6-311++G (d,p) level of theory with SMD solvation (THF). All energies are reported in kcal/mol and referenced to the lowest energy carbanion generated after initial deprotonation. Figure and calculations obtained from Dr Xiang Li.

2.3.4 Transamidation

The tetramate esters **25a-d** were further functionalised by transamidation reactions with primary amines to give carboxamides **27-30(a-d)**. Ethyl esters are not considered as activated species for amide bond formation and the extended conjugation observed in the tricarbonyl system in tetramate esters adds further difficulty in the reaction.¹¹⁰ Some reactions did not go to completion by refluxing in THF/toluene for 18 h and yields varied (10 - 70%, Table 2.4), probably as a result of multiple factors such as solubility of tetramate esters, steric hindrance and ease of purification by column chromatography. The relative *trans* stereochemistry was expected to be conserved during the course of reaction with characteristic H-2 and H-5 chemical shift data (Table 2.4). This *trans* stereochemistry was further supported by NOE analysis of **30d**, indicating an enhancement between H-5 and aromatic H-2' protons (Figure 2.12). The absolute stereochemistry was confirmed previously by X-ray crystallography for one tetramate carboxamide **21v** (Figure 2.13).¹¹⁰ Given that transamidation proceeded under the same reaction condition and the characteristic chemical shifts were comparable between both series,¹¹⁰ the stereochemistry in the newly generated tetramate series with C-2 pyridine rings was assigned as such.

Table 2.4 Yield, H2 and H5 chemical shifts (CDCl₃, 400 MHz) for tetramate carboxamides.



	R ² =	R ¹ =	δ_{H} (ppm)		% Yield
			H-2	H-5	
27a			6.35	5.11 (br. s)	70
27b			6.18	4.81 (app t)	52
27c			6.28	5.08 (app t)	62
27d			6.24	4.82 (app t)	67
28a			6.33	4.84 (br. s)	41
28b			6.16 (major)	4.61 (major, br. s)	15
			6.24 (minor)	4.73 (minor, br. s)	
28c			6.25	4.81 (br. s)	31
28d			6.24	4.59 (br. s)	24
29b			6.14 (major)	4.67 (major, br. s)	12
			6.20 (minor)	4.39 (minor, dd)	
29c			6.30 (major)	4.90 (major, app t)	10
			6.25 (minor)	-	
30a			6.31	4.84 (br. s)	44
30b			6.17	4.48 (br. s)	33
30c			6.25 (major)	4.81 (app t)	38
			6.33 (minor)	4.57 (app t)	
30d			6.23 (major)	4.59 (app t)	35
			6.31 (minor)	-	

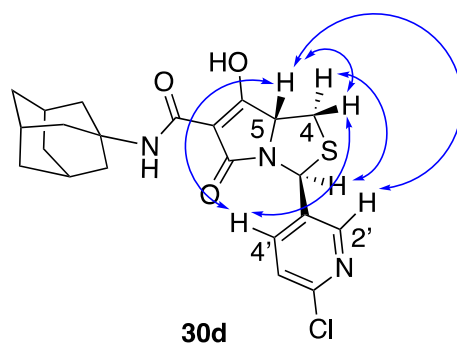


Figure 2.12 NOE analysis of tetramate carboxamide **30d**.

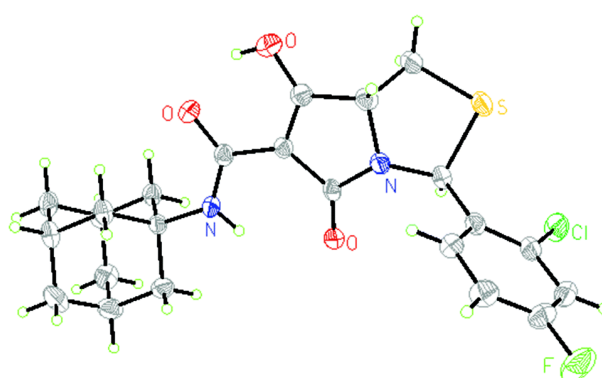


Figure 2.13 Reported structure of tetramate carboxamide **21v** from single crystal X-ray diffraction studies. Reprinted with permission from the Royal Society of Chemistry (Panduwawala, T. D.; Iqbal, S.; Thompson, A. L.; Genov, M.; Pretsch, A.; Pretsch, D.; Liu, S.; Ebricht, R. H.; Howells, A.; Maxwell, A.; Moloney, M. G. *Org. Biomol. Chem.* **2019**, *17*, 5615–5632.) Copyright 2019.

^1H NMR spectra for tetramate carboxamides indicated complete enolisation, with no chemical shift for H-7 detected. Tautomeric behaviour was known to exist for 3-acyltetramic acids in solution.^{63,110} As shown in Figure 2.14 for tetramate carboxamides, tautomeric pairs **AB** and **CD** are internal tautomers which rapidly exchange with each other through proton transfer.⁶³ Averaged out signals were observed for **AB** and **CD** on NMR time scale.⁶³ Only the external tautomerism, between the endo-enolic pair **A/C** and

between the exo-enolic pair **B/D**, can be detected on NMR spectroscopy, with distinct ^1H and ^{13}C chemical shifts.⁶³ The assignment of the chemical shifts for carbonyl functional groups C-6, C-8 and C-9 was supported by HMBC analysis (Figure 2.15), which also confirmed the assignment of the tautomeric forms. By tabulation of the chemical shifts of H-2 and carbonyl carbons (C-6, C-8, C-9), similar pattern for the major/minor tautomers was observed across the series (Table 2.5), which was also consistent with that observed in previously reported tetramate carboxamides with C-2 aromatic rings.¹¹⁰ The X-ray structure of the aforementioned tetramate carboxamide **21v** also confirmed the major tautomer as the endo-enolic form **A** (Figure 2.13, page 58, *vide supra*).¹¹⁰

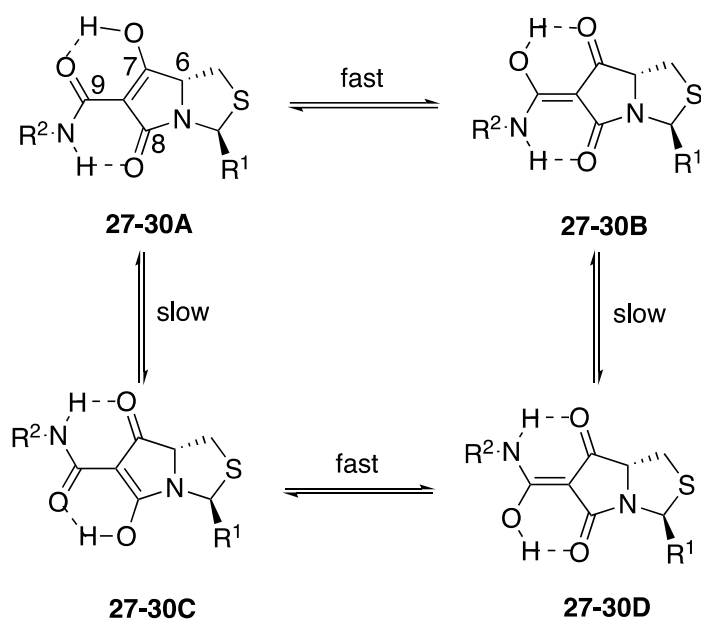


Figure 2.14 Tautomeric behaviour of tetramate carboxamides (dotted line suggests potential intramolecular hydrogen bonding). Adapted with permission from the Royal Society of Chemistry (Panduwawala, T. D.; Iqbal, S.; Thompson, A. L.; Genov, M.; Pretsch, A.; Pretsch, D.; Liu, S.; Ebricht, R. H.; Howells, A.; Maxwell, A.; Moloney, M. G. *Org. Biomol. Chem.* **2019**, *17*, 5615–5632.) Copyright 2019.

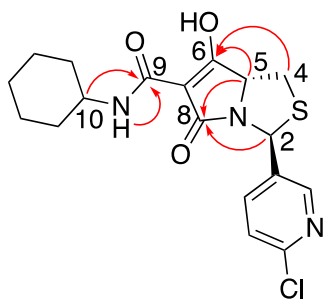


Figure 2.15 HMBC analysis of tetramate carboxamide **28d**. C-6 correlated with H4 and H-5 protons; C-8 correlated with H-2 and H-5 protons; C-9 correlated with H-10 and NH protons.

Table 2.5 H-2 and C-6, C-8, C-9 chemical shifts (CDCl₃) of tetramate carboxamides.

	R ² =	R ¹ =	δ (ppm)				AB/CD ^a	
			H-2	C-6	C-8	C-9		
27a			6.35	188.0	-	163.8	AB only	
27b			6.18	184.9	172.3	163.6	AB only	
27c			6.28	185.0	171.9	163.7	AB only	
27d			6.24	184.7	172.3	163.7	AB only	
28a			6.33	-	-	165.5	AB only	
28b			AB	6.16	189.1	172.8	165.4	3.3 : 1
			CD	6.24	191.2	178.4	-	
28c			6.25	188.5	172.3	165.4	AB only	
28d			6.24	188.7	173.3	165.4	AB only	
29b			AB	6.14	187.2	172.5	165.3	3.3 : 1
			CD	6.20	193.0	176.9	165.9	
29c			AB	6.30	177.9	173.0	167.9	3.4 : 1
			CD	6.25	-	-	-	
30a			6.31	188.8	-	166.3	AB only	
30b			6.17	190.0	-	166.2	AB only	
30c			AB	6.25	189.2	172.5	166.2	2.3 : 1
			CD	6.33	-	-	-	
30d				AB	6.23	189.5	173.0	166.2
	CD			6.31	191.1	-	167.9	

[a] The tautomeric ratio **AB/CD** was calculated as the ratio of integration of H-2. – in most cases where sample mass was low, routine ¹³C NMR did not seem to be sensitive enough to detect all ¹³C signals for minor tautomers.

2.4 Metal chelating properties of tetramates

The purification of tetramate esters and carboxamides by flash silica column chromatography has been reported to result in broadening of signals in ^1H NMR spectra, which was attributed to the formation of metal chelates during the process where silica gel and sand contained trace amounts of metal impurities such as Mg^{2+} , Fe^{2+} and Ca^{2+} .^{106,109,110} Metal free tetramates with improved signal sharpness and resolution were obtained by running the chromatography column with 1% Et_3N and subsequently washing with 5% citric acid.^{109,110} The presence of a basic pyridine ring ($\text{pK}_a = 5.23$) in the tetramate systems limited the acid strength for post-column wash and also inevitably resulted in loss of yield to the acidic aqueous layer.

Attempts were made to characterise the type of metal ions present in metal chelates by mass spectrometry. Unfortunately, no plausible peaks for metal-organic complexes could be detected. The signal broadening of signals on ^1H NMR was thus used as a rough qualitative estimate of metal chelating abilities of tetramate derivatives. After characterisation of the post-column and acid-washed compound by ^1H NMR spectrometry, metal-free sample of tetramate ester **25** was re-dissolved in DCM and deliberately washed with discrete 1M aqueous solutions of metal ions (Figure 2.16). By comparing the set of ^1H NMR spectra, the relative metal binding abilities and the relative affinities to specific metal ions were better understood. Tetramate derivatives exhibited structure-dependent metal binding properties; the tetramate carboxamides appeared to bind more strongly with metals than tetramate esters (Figure 2.16 A versus E). They also demonstrated a higher affinity to bind with certain metal ions, such as Fe^{2+} as compared to Ca^{2+} (Figure 2.16, C versus D).

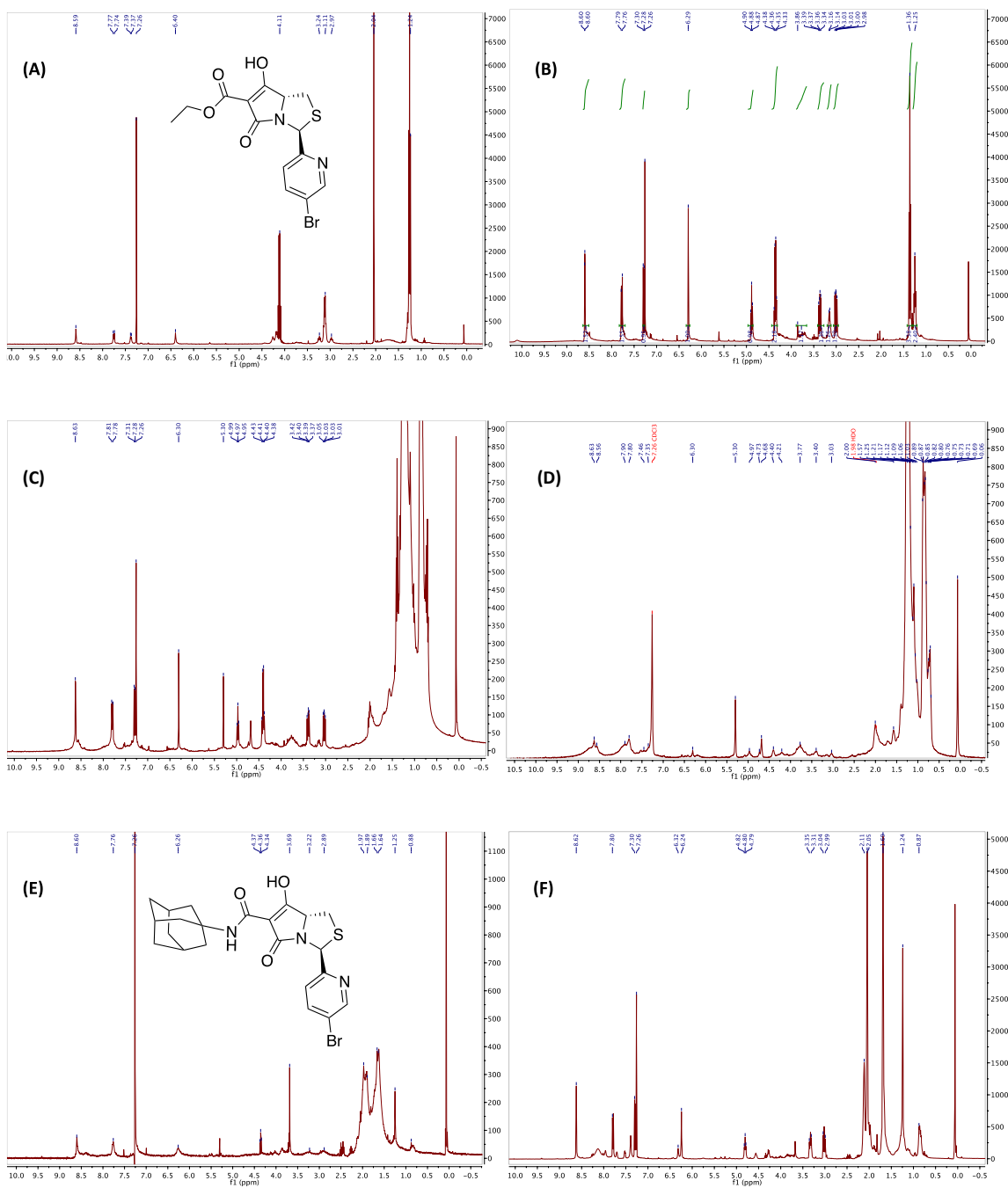
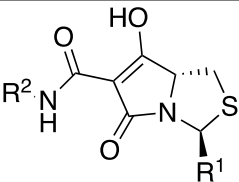
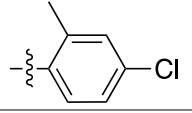
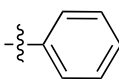
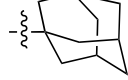
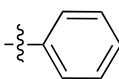
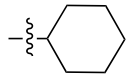
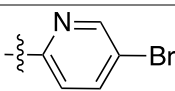
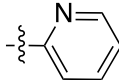
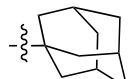
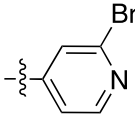
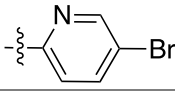
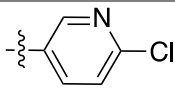


Figure 2.16 ^1H NMR (CDCl_3 , 400 MHz) spectra indicating metal-chelation abilities of tetramate ester **25c** and tetramate carboxamide **30c**. (A) Tetramate ester **25c** post-column, before acidic wash; (B) Tetramate ester **25c** post-column, after 5% citric acid wash; (C) Metal-free **25c** washed with 1M aq. $\text{Ca}(\text{NO}_3)_2$ solution; (D) Metal-free **25c** washed with 1M aq. FeSO_4 solution; (E) Tetramate carboxamide **30c** post-column, before acidic wash; (F) Tetramate carboxamide **30c** post-column, after 5% citric acid wash.

2.5 Biological evaluation

The tetramates esters **25a-d** and carboxamides **27-30(a-d)** were tested for their biological activities against Gram-positive methicillin-resistant *Staphylococcus aureus* (MRSA) and Gram-negative *Escherichia coli* (*E. coli*) by minimum inhibitory concentration (MIC) assays. No activity was seen against the Gram-negative *E. coli*. None of the tetramate esters **25(a-d)** showed any activity. A total of five tetramate carboxamides were tested active against MRSA with MIC values < 8 µg/ml (Table 2.6). Primary evaluation indicated that a hydrophobic pendant at C-9 was essential for bioactivity while substitutions with heterocycles at C-2 successfully increased compound polarity without compromising bioactivity. Unfortunately, these tetramate analogues were again rendered inactive when tested in the presence of blood, suggesting the presence of other blood-associated factors affecting bioactivity besides plasma protein binding. A more detailed analysis of structure-activity relationship is described in Chapter 5.

Table 2.6 Biologically active cysteine-derived tetramate analogues.

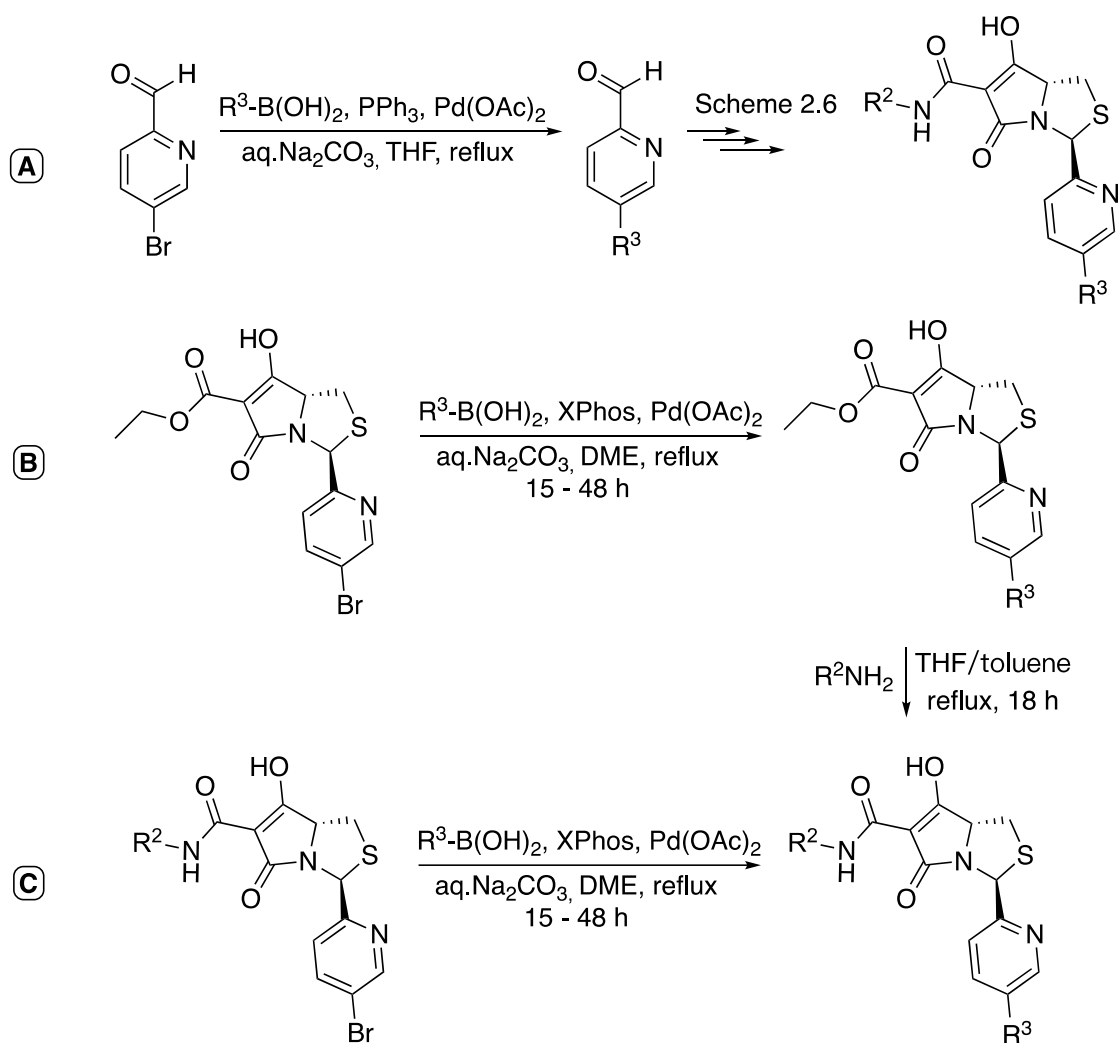
	R ² =	R ¹ =	MIC against MRSA μg/ml	MW	clogP ^a	PSA ^a /Å ²
GM018			3.91 - 7.81 ^b	401	3.21	69.6
GM022			0.98 - 3.91 ^b	411	1.78	69.6
28c			7.81	438	1.41	82.5
30a			1.95	412	0.75	82.5
30b			1.95	490	1.66	82.5
30c			3.91	490	1.54	82.5
30d			0.24	446	1.36	82.5

[a] Relevant chemical properties of the active compounds were calculated using MarvinSketch 20.3.0, 2020, ChemAxon. [b] MIC values determined with two different strains of MRSA.

2.6 Suggestion for future work

The presence of halogens in C-2 heterocycles offers opportunities for further elaboration of C-2 substituents through Suzuki-Miyaura cross coupling reactions (Scheme 2.10). The diversity of accessible boronic acid derivatives and the possibility of late-stage diversification allow further fine modulation of polarity through C-2 functionalisation.¹¹⁰ Late-stage functionalisation by Suzuki-Miyaura coupling in cysteine-derived tetramate systems failed under traditional coupling conditions as both the 1,3-bicarbonyl system

and thioether of the derivatives can chelate with metals, potentially inactivating the palladium Pd(0)/Pd(II) catalyst.¹¹⁰ Prevention of catalyst deactivation was however possible through the use of dialkylbiaryl phosphine ligands (e.g. XPhos), with increased electron density at phosphorous and subsequently at Pd(0) in the monoligated species, instead of the conventional triphenyl phosphine (PPh₃) in the coupling reactions.^{110,131,132}



Scheme 2.10 Possible elaboration of C-2 substituents through Suzuki-Miyaura coupling reactions (reaction time can be shortened with microwave irradiation). (A) direct coupling on aldehyde; (B) coupling on tetramate esters; (C) coupling on tetramate carboxamides.

2.7 Summary

This chapter described the synthesis of cysteine-derived bicyclic tetramate analogues with C-2 and C-7 substitutions. The aim of designing target tetramate library was to decrease plasma protein binding by increasing compound polarity. Tetramates derived from L-cysteine were more stable and allowed greater variations of condensing species than corresponding systems derived from L-serine and L-threonine. The synthetic pathway was highly chemoselective and stereoselective, leading to bicyclic tetramate esters and tetramate carboxamides with a *trans* relationship between H-2 and H-5. The Dieckmann cyclisation proceeded in the same direction to that previously observed in C-2 aromatic series, but different to that in the C-2 *t*-butyl and isopropyl series, highlighting the steric control exerted by C-2 substituents on the chemoselectivity of the cyclisation process. The synthetic goal of increasing polarity of tetramate carboxamides was achieved by introducing heterocycles at C-2 and C-9 amide pendant. While the tetramate template can tolerate an increase in polarity by C-2 functionalisation without diminishing *in vitro* antibacterial activity, tetramate analogues with polar C-9 pendants were inactive, suggesting C-9 amide pendant was not a suitable position for polarity modulation. Further fine-tuning of polarity can be achieved by Suzuki-Miyaura coupling on halogen-substituted heterocycles at C-2. Tetramate analogues exhibited structure dependent metal chelation properties, influencing compound purification and biological activities. Despite the increase in polarity and resultant decrease in plasma protein binding, these cysteine-derived tetramate analogues lost their potent *in vitro* bioactivity when tested in the presence of blood. This suggested that other factors besides plasma protein binding were implicated in bioactivity. Metal binding ability was hypothesised to be one such factor and efforts to modify metal chelation were detailed in the following two chapters.

Chapter 3 Modification of metal chelation in cysteine-derived tetramate systems - I

“The intrinsic metal-chelating property of 3-acylated tetramate acids (and tetronic acids) is a reality that should be systematically taken into account when studying their biological effects from molecular to cellular levels.”⁶¹

— Mehdi Zaghouani, 2016

This chapter describes the synthesis of cysteine-derived tetramate analogues with functionalisation at C-6 enol and C-9 carbonyl groups (Figure 3.1). The aim of functionalisation was to modify the functional groups directly involved in metal chelation of the tetramate system to alter its metal binding ability. During characterisation of the synthesised tetramate library, an interesting C-7 alkylation leading to a C-7 quaternary centre was observed. The C-7 alkylated tetramate analogues, with apparently no metal binding ability, were found to be active against MRSA, thus warranting further optimisation.

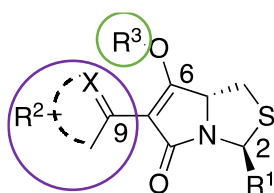


Figure 3.1 Cysteine-derived tetramate analogues with C-6 and C-9 functionalisation.

3.1 Introduction

It was observed that cysteine-derived tetramate analogues demonstrated excellent antibacterial activities against MRSA, but which diminished when tested *in vitro* in the presence of blood (Table 1.6, page 28, *vide supra*). The reduction in bioactivity was hypothesised to be associated with plasma protein binding and/or metal chelation. Chapter 2 has described approaches to decrease plasma protein binding by increasing compound polarity, yet these changes unfortunately have not effectively prevented activity loss when tested in the presence of blood. This chapter and the following chapter therefore focus on approaches to modulate metal chelation in cysteine-derived tetramate analogues, so as to investigate its effect on the antibacterial activity.

Naturally occurring 3-acyltetramates, with distinct antibacterial activities, were reported to exhibit metal-binding abilities.^{47,59-61} The mode of metal complexation of 3-acyltetramate acids is shown in Figure 3.2. Depending on the coordinated metal ion, 3-acyltetramate acids form metal chelates either in the *E* or *Z* configuration (Figure 3.2).⁴⁷ For example, the X-ray crystal structure of a Cu(II) chelate of a 3-acyltetramic acid revealed that it existed in a *Z*-enol square planar complex (Figure 3.3 A).^{133,134} Group II metal ions such as Mg(II) and Ca(II) were reported to form complexes of the same *Z*-configuration, supported by virtually identical IR spectra.¹³⁵ IR analysis showed that an Fe(III) complex of the same tetramic acid as shown in Figure 3.3 A existed mainly in the *Z*-form while its Ni(II) complex mainly in its *E*-form.¹³⁴ Various other metal chelates of 3-acyltetramates have also been reported such as Zn(II), Ga(III), La(III), Ru(II),¹³⁶ Al(III),¹³⁷ Pd(II) (an example of metal chelate in *E*-configuration as shown by its crystal structure, Figure 3.3 B),¹³⁸ Co(II),¹³⁹ Rh(I),¹⁴⁰ Na(I), K(I),¹⁴¹ Cs(I),¹⁴² Cd(II), Pb(II),¹⁴³ Ba(II),¹⁴⁴ Hg(II),¹⁴⁵

and Mn(II).¹⁴⁶ It was also noted that in amorphous solid state or in solution, metal chelates of tetramic acids might exist in a mixture of the two possible configurations.¹³⁴

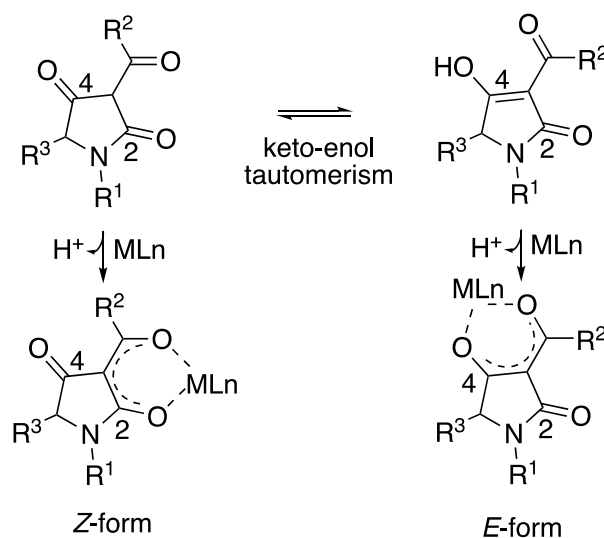


Figure 3.2 Models of metal complexation in 3-acyltetramic acids. Adapted with permission from Expert Opinion on Drug Discovery (Dandawatea, P.; Padhyeb, S.; Schobert R.; Biersack, B. *Expert Opin. Drug Discovery* **2019**, 14 (6), 563-576). Copyright (2019) Taylor & Francis.

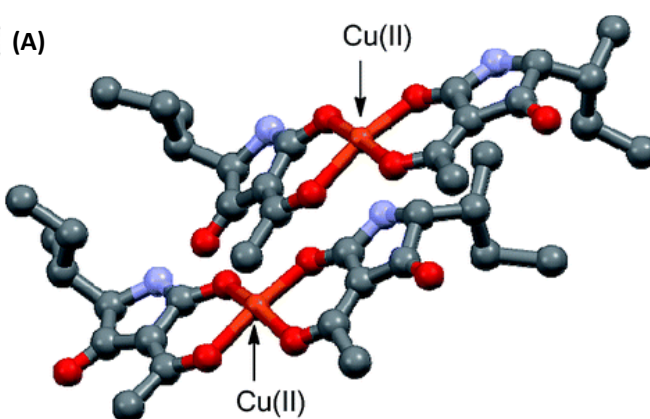


Figure 3.3 (A) X-ray crystal structure of Cu(II) chelate of a 3-acyltetramic acid in Z-configuration. Adapted by permission of The Royal Society of Chemistry (Zaghouani, M.; Nay, B. *Nat. Prod. Rep.* **2016**, 33, 540-548). Copyright (2016).

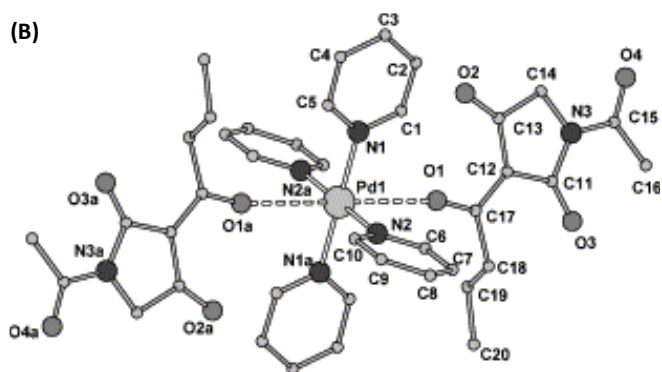


Figure 3.3 (B) X-ray crystal structure of Pd(II) chelate of a 3-acyltetramic acid in *E*-configuration. Reprinted with permission from *Inorganica Chimica Acta* (Gavrielatos, E.; Athanasellis, G.; Heaton, B. T.; Steiner, A.; Bickley, J. F.; Igglessi-Markopoulou, O.; Markopoulos, J. *Inorg. Chim. Acta* **2003**, 351, 21-26). Copyright (2003) Elsevier B.V.

The tetramate library synthesised by the Moloney group has been reported to exhibit metal chelating properties as indicated by post-column broadening of ^1H NMR spectra.^{106,109,110,112} X-ray photoelectron spectroscopic (XPS) analysis of previously synthesised tetramate derivatives (post-column) identified the presence of Ca.¹¹² The high abundance of Ca, together with Na, Mg, Fe and Zn, were also found in metal-chelated tetramates by inductively coupled plasma mass spectrometry (ICP-MS).¹¹⁰ By comparison of ^1H NMR spectra of post-column, acid-washed and metal-chelated tetramates, metal complexation behaviour of tetramates derivatives was also found to be structure dependent and metal-ion dependent (Figure 2.16, page 63, *vide supra*). Given the heterogeneity of metal ions participating in chelation and the likelihood of tautomeric equilibrium in solution, the C-6 enolic oxygen, C-8 amide carbonyl and C-9 carbonyl of the tetramate derivatives were all likely to be the sites of metal complexation.

There is a complex relationship between metal chelation of tetramate acids and their biological activities.^{47,60} Metal sequestration was found to be one of the modes of antibacterial action of tetramate acids, suggesting metal binding ability was essential for their biological activities.^{47,147} Metal chelates of a 3-acyltetramic acid, melophlin C with Ga(III), La(III) and Ru(II), all of which were considered Fe(III) mimics, were found to be active against *Micrococcus luteus* (*M. luteus*).¹³⁶ Interestingly, the antibacterial activity was not observed with the metal free melophlin C.¹³⁶ Streptolydigin (Figure 1.6, page 14, *vide supra*), a tetramate-containing natural product, was reported to inhibit bacterial RNA polymerase (RNAP) but not eukaryotic RNA polymerases; non-catalytic Mg(II) ion present in bacterial RNAP was found to be essential for its binding,¹⁴⁸ indicating that metal chelation is not only important for the potency and efficacy of tetramate derivatives, but also for their selectivity and safety as antibacterial agents. Some tetramic acids demonstrated increased toxicity as a metal complex such as magnesidin,¹³⁵ while in others, toxicity may be attenuated by metal chelation.¹⁴⁹ On the other hand, aqueous solubility and stability of metal complexes of tetramate derivatives were also reported to influence their biological activity.^{47,60} The tetramate acid shown in Figure 3.3 A (page 70, *vide supra*) was found to bind weakly with Cu(II) with the resultant complex being unstable in water.¹³⁴ In contrast, it formed a strong and stable chelate with Fe(III), which was poorly soluble in water.¹³⁴ Similarly, tetramate carboxamides **27-30(a-d)** synthesised previously were found to precipitate out of aqueous solution upon titration of Fe(III) solution. Metal chelates of tetramic acids generally exhibit greater lipophilicity than the free acid forms, therefore impacting aqueous solubility and permeability through cell membrane.⁶¹ The aqueous solubility of metal complexes of tetramate derivatives could determine their effective concentration delivered to the site of bacterial infections; the

polarity of metal complexes could affect its permeability across bacterial cell membrane; the stability of metal complexes could influence the availability of tetramate derivatives to interact with target enzymes, all of which could contribute to their observed biological activities. Given the diversity of metal ions present in blood (Table 3.1)¹⁵⁰ and various factors associated with metal chelation and biological activities, an overall phenotypic assay was employed for assessing antibacterial activities of the tetramate library. Rather than unidirectionally increasing or decreasing metal chelation abilities through chemical modifications, this study aimed to access the sites involved in metal chelation to modulate the property, and where possible, to design tetramate analogues as prodrugs, which would solve the problem of metal chelation during transport and permeation, potentially address bioavailability, and restore biological activity when in a whole organism assay.

Table 3.1 Clinical reference values of metal ions in human blood of healthy adults.¹⁵⁰

Types of metal ions		Laboratory reference range
Calcium (Age-dependent)		2.20 – 2.60 mmol/L
Iron	Male	13 – 31 $\mu\text{mol/L}$;
	Female	5 – 29 $\mu\text{mol/L}$
Magnesium		0.65 – 1.05 mmol/L
Potassium		3.5 – 5.1 mmol/L
Sodium		135 – 145 mmol/L
Lithium		0.5 – 0.8 mmol/L
Zinc		10 – 18 $\mu\text{mol/l}$

The synthetic goal of this chapter was thus to alter metal chelation ability of tetramate analogues by modification of the functional groups expected to be directly participating in metal binding – and particularly C-9 carbonyl and C-6 enol groups, because of their relative ease of chemical accessibility over C-8 amide carbonyl group. The C-9 carbonyl

group was first replaced with a C=N bond (Figure 3.4 A). The hypothesis underlying this choice was that the resultant tetramate analogue would have reduced metal binding ability with N being less electronegative than O and C-6 enol being less acidic. A second point change in the tetramate structure was introduced through C-6 enol functionalisation (Figure 3.4 B). The acidic proton at C-6 enol was replaced by 1) small substituents, with increased steric bulkiness such as methyl, ethyl and isopropyl groups; 2) allyl and prenyl groups, with increased stability of the corresponding carbocations and hence increased likelihood of generating the parent tetramate analogues under physiological conditions; 3) with tetrahydropyran (THP) group, bearing structural similarity to sugar derivatives and 4) with short chain linkers with further possibility of functionalisation. The eventual aim of C-6 functionalisation was to synthesise tetramate analogue as a prodrug with a C-6 sugar derivative, which both blocks metal chelation during transport in bloodstream and serves as a recognition motif for bacterial cellular uptake, and when cleaved at the glycosidic bond by bacterial glycoside hydrolases, produces the active parent tetramate. This also corresponded to the idea of formulating tetramate-based prodrugs that act as “Trojan horses”, which ‘trick’ bacteria for drug intake as nutrients through specific transport channels on bacterial cell membrane and ‘wreak havoc’ due to their reported biological mechanism of metal sequestration, scavenging and deprivation of essential metal ions from bacterial cells.^{47,147}

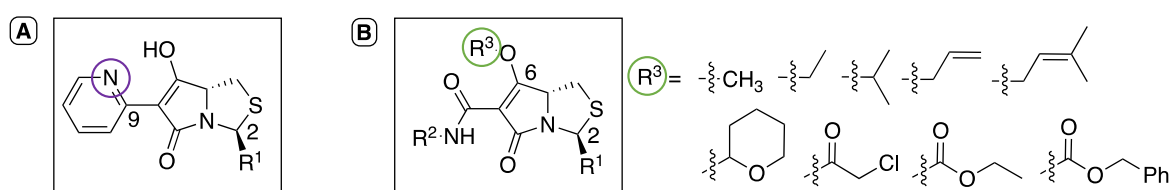
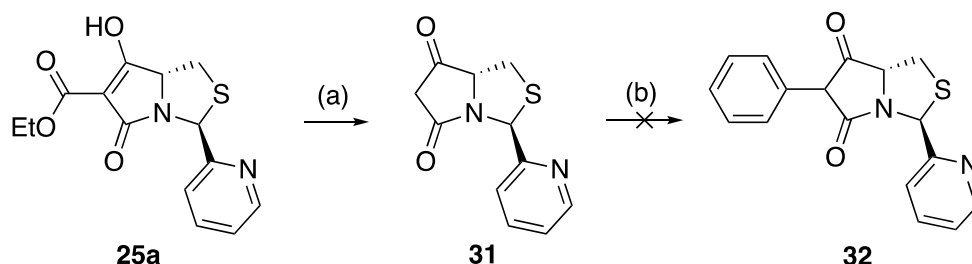


Figure 3.4 Target library of cysteine-derived tetramate analogues.

3.2 Synthesis of model tetramate-based compound with a C-9 C=N bond

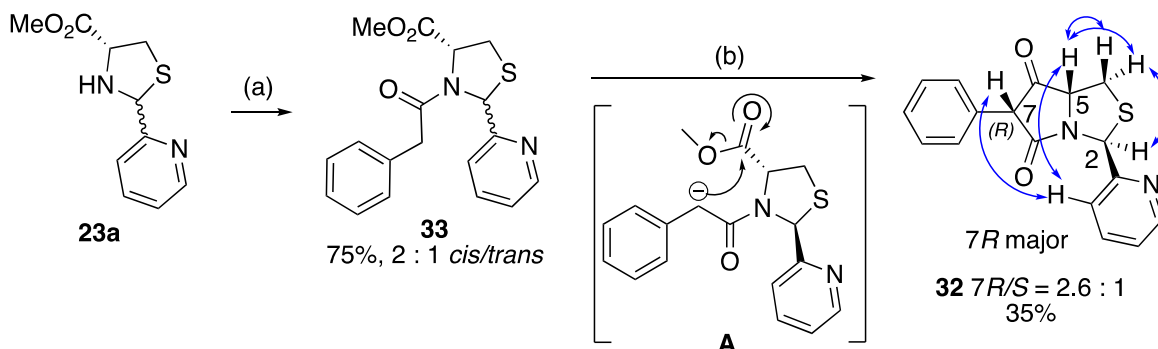
In order to substitute the C-9 carbonyl group, a synthetic route with late-stage functionalisation of the synthesised tetramate core was first devised (Scheme 3.1). In this route, the major cysteine-derived tetramate ester product from Dieckmann cyclisation **25a** was successfully decarboxylated to a C-7 unsubstituted tetramate derivative **31** under a previously reported procedure.¹⁵¹ The resulting tetramate analogue **31**, with acidic C-7 protons, was subject to the specific arylation conditions with Pb(IV) tetraacetate, known to be highly effective in simpler substrates.^{152,153} However, the coupling failed with the reaction mixture turning black and starting material being partially recovered, suggesting catalyst inactivation by the metal-binding thioether group present in the tetramate analogue.



Scheme 3.1 Attempted synthesis of tetramate analogue with C-7 aryl-substituent from tetramate ester **25a**. *Reagents and conditions:* (a) LiOH monohydrate, THF/H₂O (1:1), r.t., o.n.; (b) Pb(IV) tetraacetate, phenylboronic acid, CHCl₃, 40 °C, 1 h, followed by addition of pyridine in CHCl₃, heat at 40 °C for 1 h, cooled to r.t., o.n.

Modification was therefore carried out in the previous *N*-acylation step, one step before the formation of the bicyclic tetramate system. Under the DCC-DMAP coupling conditions, the original monoethyl malonate was replaced with an acetic acid, which forced the

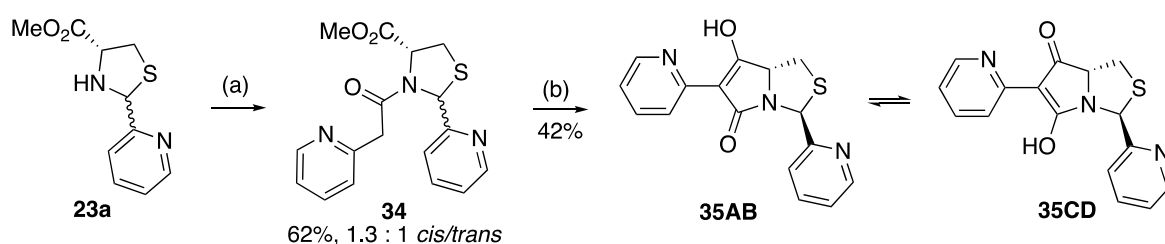
subsequent Dieckmann cyclisation to go in one direction, closing from the side chain enolate to the C-5 methyl ester (enolate type A, Scheme 3.2) to form a stable 5-membered ring. The synthetic route was first tested with phenylacetic acid (Scheme 3.2), where *N*-acylation resulted in the formation of *N*-acylthiazolidine **33** as a 2 : 1 mixture of *cis/trans* diastereomers, each as a pair of rotamers, in 75% yield and Dieckmann cyclisation led to the formation of a bicyclic tetramate analogue **32** with a C-7 phenyl substituent in 35% yield.^a The tetramate analogue **32** existed predominantly in the keto-form in organic solvent (CDCl₃), by observation of the C-7 protons in ¹H NMR spectrum with keto/enol tautomeric ratio of 7 : 3. The keto form was a 2.6 : 1 7*R*/*S* mixture of diastereomers with a *trans*-relationship between H-2 and H-5, where the relative stereochemistry was confirmed by NOE analysis (Scheme 3.2).



Scheme 3.2 Synthesis of tetramate analogue **32** (NOE analysis shown with blue arrows to confirm the relative stereochemistry) with modification of the *N*-acylation step. *Reagents and conditions*: (a) phenylacetic acid, DCC, DMAP, DCM, 0 °C to r.t., 18 h; (b) KO^tBu, THF, reflux, 4 h.

^a To place the C-2 substituent at the less hindered *exo* face of the bicyclic ring, only the *trans* diastereomer of **33** that cyclised so that the maximum theoretical yield of **32** would be 33%. The experimental yield of 35% suggested a potential for reduced purity, shift in *cis/trans* equilibrium of **33** that resulted in dynamic kinetic resolution or epimerisation at C-2 and C-5.

The synthetic route was repeated with 2-pyridylacetic acid, which was stabilised as a HCl salt, to bring a C=N bond at C-9 position of the tetramate product (Scheme 3.3). Given the increased polarity of pyridylacetic acid HCl salt, its insolubility in the original reaction using DCM solvent hindered the *N*-acylation reaction with no product isolation. Addition of Et₃N to the reaction mixture to neutralise the HCl salt did not result in an improvement in the reaction outcome. The coupling condition was thus changed in T₃P/DIPEA in the much more polar DMF solvent, which successfully produced the *N*-acylthiazolidine **34** as a 1.3 : 1 mixture of *cis/trans* diastereomers, each as a pair of rotamers, in 62% yield. Subsequent Dieckmann cyclisation resulted in the desired tetramate analogue in 42% yield.^a In this case, the tetramate derivative showed complete enolisation, since no C-7 proton was observed and it existed as an approximately 1 : 1 mixture of AB : CD tautomers with a *trans*-relationship between H-2 and H-5, where the relative stereochemistry was confirmed by NOE analysis (Figure 3.5).



Scheme 3.3 Synthesis of target tetramate analogue **35** with C-9 C=N bond instead of a carbonyl group. *Reagents and conditions:* (a) 2-pyridylacetic acid HCl, T₃P, DIPEA, anhydrous DMF, 0 °C to r.t., 18 h; (b) KO^tBu, THF, reflux, 4 h.

^a Similar to *N*-acylthiazolidine **33**, it was the *trans* diastereomer of **34** that cyclised predominately to place the bulky C-2 pyridyl ring at the less hindered *exo* face of the bicyclic system. The maximum theoretical yield of **35** would be 43%, discounting potential dynamic kinetic resolution or epimerisation at C-2 and C-5.

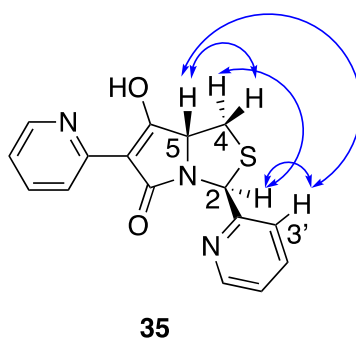


Figure 3.5 NOE analysis of target tetramate analogue **35**.

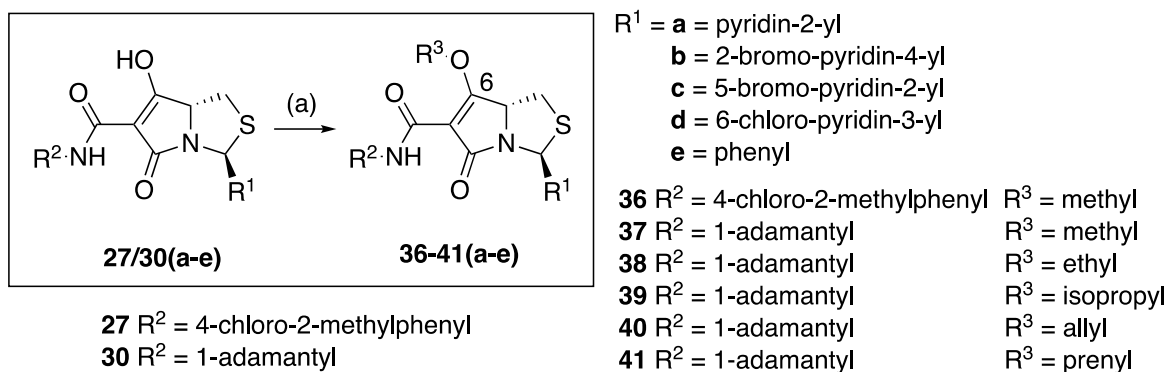
The versatility of this synthetic route to produce tetramate analogues with a C=N bond in replacement of C-9 carbonyl group was limited by the availability of the corresponding acetic acids in the *N*-acylation step. Instead of producing a series of tetramate derivatives with C-9 C=N bond with its electronic nature potentially modulated by various aryl-substituents, the model compound **35** was first studied for its metal chelation ability and tested for its antibacterial activity (see Section 3.4 and 3.5).

3.3 C-6 Hydroxyl group functionalisation

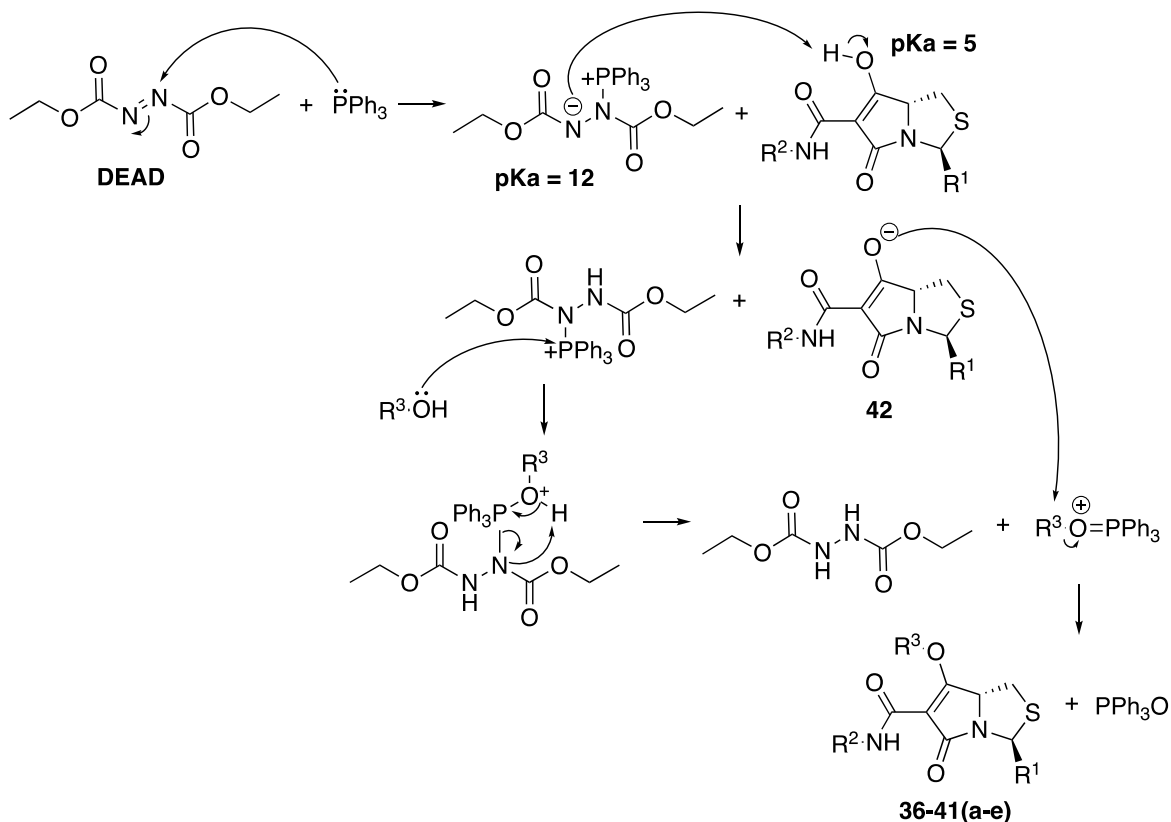
3.3.1 Alkylation under the Mitsunobu conditions

Direct alkylation of tetramate ester **25c** and tetramate carboxamide **30e** by reactions with alkyl halides under basic conditions has been completely unsuccessful, probably due to the low nucleophilicity of the C-6 hydroxyl group on these systems. The C-6 enol group, with its acidity conferred by the extended tricarbonyl conjugated system, could be functionalised with respective alcohols under Mitsunobu conditions (Scheme 3.4). According to the proposed Mitsunobu mechanism, the tetramate analogue was first deprotonated at the C-6 hydroxyl group by the DEAD-PPh₃ adduct; the hydroxyl group of the incoming alcohol was subsequently converted into a better leaving group, which

eventually left as PPh₃O with an S_N2 nucleophilic attack from the deprotonated O⁻ nucleophile (Scheme 3.5).¹⁵⁴⁻¹⁵⁹ The DEAD-PPh₃ adduct has a predicted pKa of 12 while the C-6 enol functional group on the tetramate carboxamide has a predicted pKa of 5 (calculated using MarvinSketch 20.3), allowing for Mitsunobu reactions to occur.¹⁵⁹

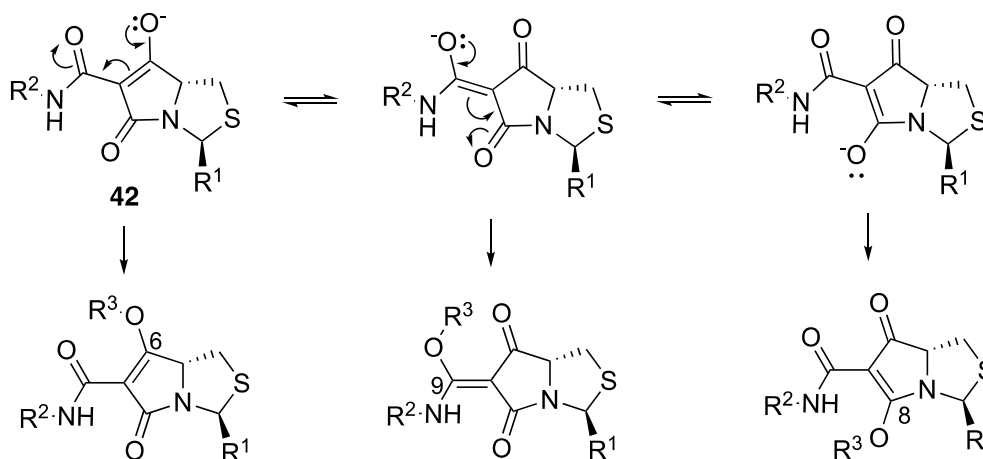


Scheme 3.4 Planned synthesis of target tetramate analogues **36-41** with C-6 enol functionalisation under the Mitsunobu conditions. *Reagents and conditions:* (a) R³OH, DEAD, PPh₃, anhydrous THF, 0 °C to r.t., 18 h.



Scheme 3.5 Proposed mechanism of the Mitsunobu reactions.

When deprotonation of the C-6 hydroxyl group occurred under the Mitsunobu conditions, the lone pair on the oxygen was delocalised in the tricarbonyl conjugated system, giving rise to resonance stabilised intermediates **42**, with the possibility of different positions of *O*-alkylation in the tetramate system (Scheme 3.6).

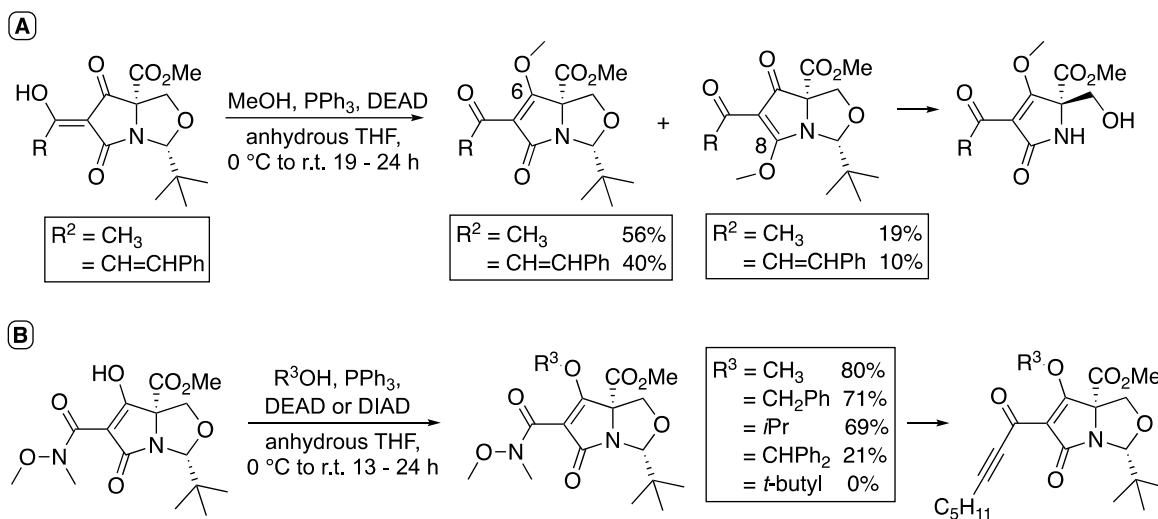


Scheme 3.6 Resonance stabilised reaction intermediate **42**, giving rise to regioisomers with different positions of *O*-alkylation.

As the reaction proceeded with an S_N2 nucleophilic attack, the scope of the reaction was limited by the strength of the nucleophile **42**, with the lone pair on the deprotonated O being extensively delocalised. The nucleophilic substitution reaction was further limited by the steric bulkiness of the reacting alcohol, with steric interactions with the bulky hydrophobic group on the C-9 amide pendant significantly reducing reaction yield.

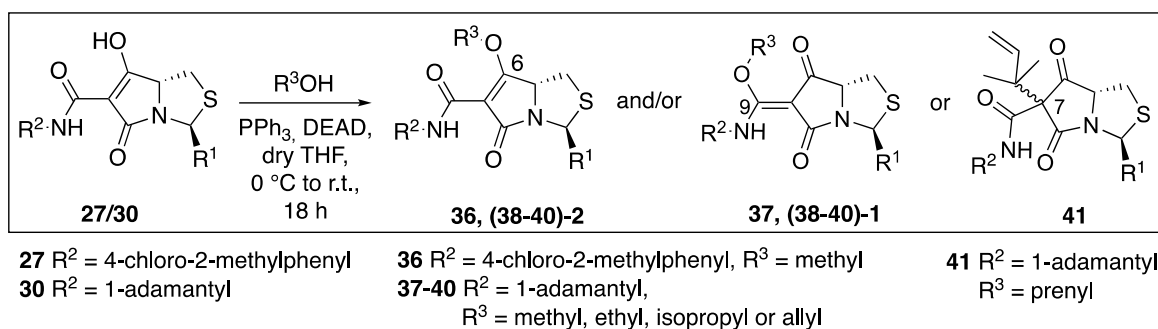
The Mitsunobu conditions has been employed previously in the Moloney group to protect the C-6 enol group of tetramate analogues for subsequent *N,O*-acetyl hydrolysis or Grignard addition of alkynes (Scheme 3.7).^{112,160} Consistent with the above predictions

from the Mitsunobu mechanism, methylation of acylated tetramate ketone analogues resulted in a mixture of C-6 *O*-alkylated and C-8 *O*-alkylated products, with the positions of *O*-alkylation confirmed by HMBC analysis (Scheme 3.7 A).¹¹² Moreover, the yield of the Mitsunobu reactions decreased with increased bulkiness of alcohols (Scheme 3.7 B).¹⁶⁰



Scheme 3.7 Previous attempts of enol protection of tetramate analogues employing the Mitsunobu conditions in the Moloney group.^{112,160}

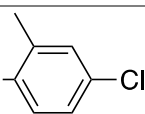

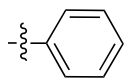
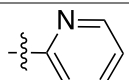


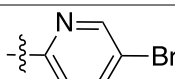
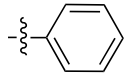
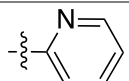
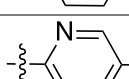

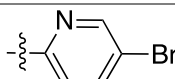

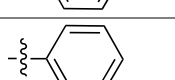
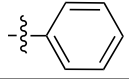
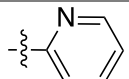
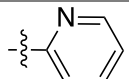

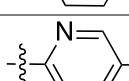
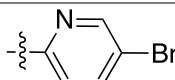

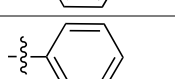
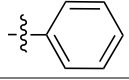
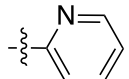
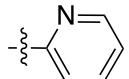

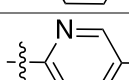
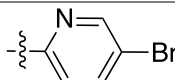


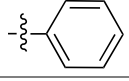
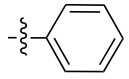
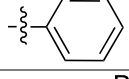

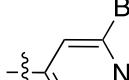

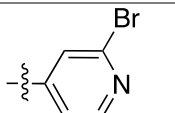
Tetramate carboxamides **36-41** were successfully synthesised under the Mitsunobu conditions employing the reactivity of the C-6 enol functionality (Scheme 3.8). A general observation from preliminary model reactions was that tetramate carboxamides were less reactive than the corresponding tetramate esters, probably due to a changed electronic nature of C-6 enols as well as the steric hindrance posed by C-9 bulky amide pendants. When benzyl alcohol was used for the reaction, no conversion was observed with recovery of the starting tetramate carboxamide. In the case of the corresponding tetramate ester **25c**, there was product formation as shown by mass spectrometry, but this product was only isolated in < 8% yield and the product was contaminated with reduced DEAD.



Scheme 3.8 Synthesis of tetramate analogues **36-41** under the Mitsunobu conditions. In cases where two regioisomers with different alkylation sites were observed from a single reaction, they were denoted by "-1" and "-2".

H-2 and H-5 chemical shifts of the alkylated tetramate carboxamides **36-41**, characteristic to a bicyclic tetramate ring, were tabulated together with the reaction yield (Table 3.2). The varied yield of the reactions and increased difficulty encountered with bulky alcohols again pointed to the unreactive nature of the C-6 enol group. The relative stereochemistry at C-2 and C-5 of the alkylated carboxamides was confirmed by NOE analysis of selected examples, where the *trans*-relationship between H-2 and H-5 was conserved from the starting carboxamides under the Mitsunobu conditions. In cases where two distinct sets of chemical shifts of products were observed from a single reaction, they did not arise from a change in the relative stereochemistry as *trans*-2,5-relationship was assigned for both isomers (Figure 3.6). Given the highly conserved H-2 and H-5 chemical shifts across the series (Table 3.2), the *trans*-2,5 stereochemistry was assigned to tetramate analogues **36-40**.

Table 3.2 Yield & H-2, H-5 chemical shifts (CDCl₃, 400 MHz) for the alkylated tetramates.

	R ² = / R ³ =	R ¹ =	δ_{H} (ppm)		% Yield	Ratio ^a
			H-2	H-5		
36e	R ² =  R ³ = 		6.33	4.88 (dd)	30	-
37a			6.49	4.57 (t)	43	-
37c	R ² =  R ³ = 		6.43	4.54 (t)	11	-
37e			6.43	4.42 (t)	31	-
38a-1			6.49	4.56 (t)	29	1 : 0.2
38a-2			6.17	Obscured ^b		
38c-1	R ² = 		6.43	4.53 (t)	17	1 : 0.2
38c-2	R ³ = 		6.10	Obscured ^b		
38e-1			6.43	4.41 (t)	32	1 : 0.1
38e-2			6.14	Obscured ^b		
39a-1			6.49	4.57 (t)	17	1 : 0.6
39a-2	R ² = 		6.29	4.85 (dd)		
39c-1			6.43	4.53 (t)	15	1 : 1
39c-2	R ³ = 		6.23	4.80 (dd)		
39e-1			6.43	4.41 (t)	27	1 : 1
39e-2			6.23	4.66 (dd)		
40a-1			6.49	4.57 (t)	5	1 : 2.8
40a-2	R ² = 		6.29	4.93 (dd)		
40c-1			6.43	4.53 (t)	11	1 : 0.2
40c-2	R ³ = 		6.24	4.71 (m)		
40e-1			6.43	4.41 (t)	21	1 : 2
40e-2			6.21	4.74 (dd)		
7R-41e			6.58	4.62 (dd)	3	1 : 5
7S-41e	R ² = 		6.55	4.33 (dd)	15	
41b	R ³ = 		6.47 6.45	4.17 – 4.27 (m)	10	1 : 1

[a] The ratio of two isomers was calculated from the isolated yield or integration ratio of H-2 chemical shifts in the isolated mixture. In the case of **40c**, a significant portion of **40c-2** was mixed with PPh₃O and therefore not isolated pure from column. [b] Obscured by the chemical shift of OCH₂CH₃, which occurred around 4.70 - 4.80 ppm.

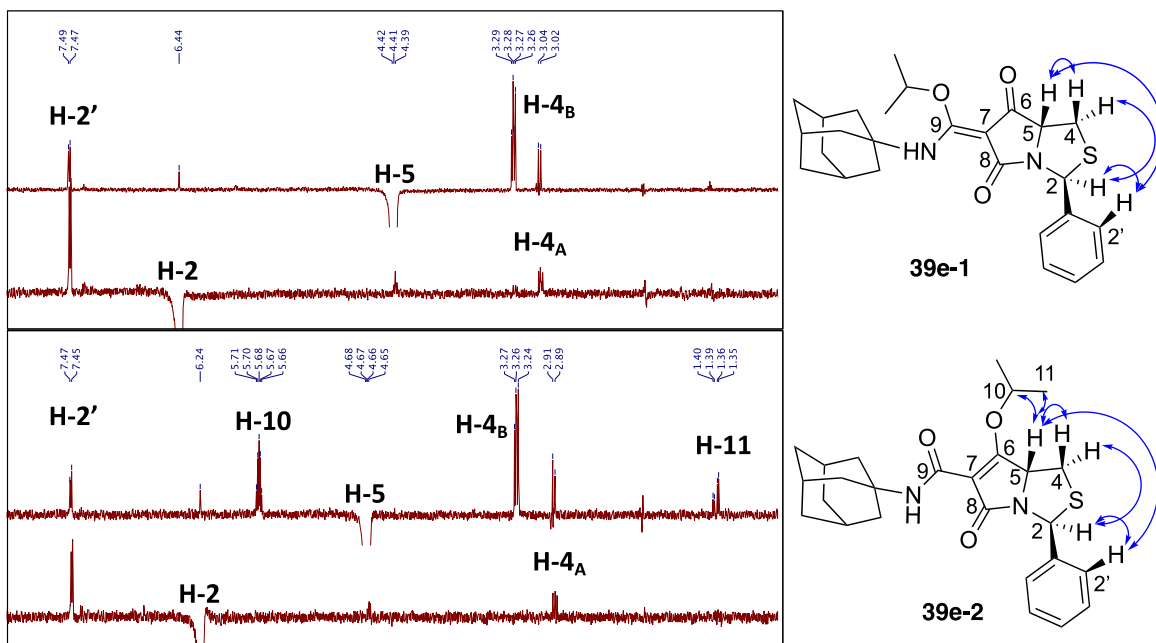


Figure 3.6 NOE analysis of alkylated carboxamide **39e-1** and **39e-2**.

The NOE analysis for **39e** also showed enhancement between H-5 and protons on the isopropyl side chain (H-10 and H-11) in **39e-2** but not in **39e-1**, suggesting that they were regioisomers with different positions of alkylation (Figure 3.6). The presence of regioisomers was further supported by tabulation of the relevant carbon chemical shifts, with generally two distinct sets of data across alkylated carboxamides (Table 3.3). In some cases, the two isomers were separable by flash column chromatography, where the distinct sets of chemical shifts did not equilibrate, confirming that they were not rotamers. The positions of alkylation were elucidated by HMBC correlation analysis (Figure 3.7). For example, in the case of **36e**, an exclusive C-6 *O*-alkylated enol ether was observed with C-6 correlated with H-4, H-5 and methoxy protons, C-8 correlated with H-2 and H-5 protons and C-9 weakly correlated with the N-H proton (Figure 3.7 A). In contrast, **37e** was a C-9 *O*-alkylated product exclusively with C-9 correlated strongly with the methoxy protons (Figure 3.7 B). In some cases of alkylated tetramate carboxamides, a large number of

scans were required to observe the C-6, C-7, C-8 and C-9 chemical shifts in ^{13}C NMR spectra; the detection and assignment of such chemical shifts were assisted by enhanced sensitivity of cryogenic ^{13}C detection probe as well as HMBC correlation analysis.

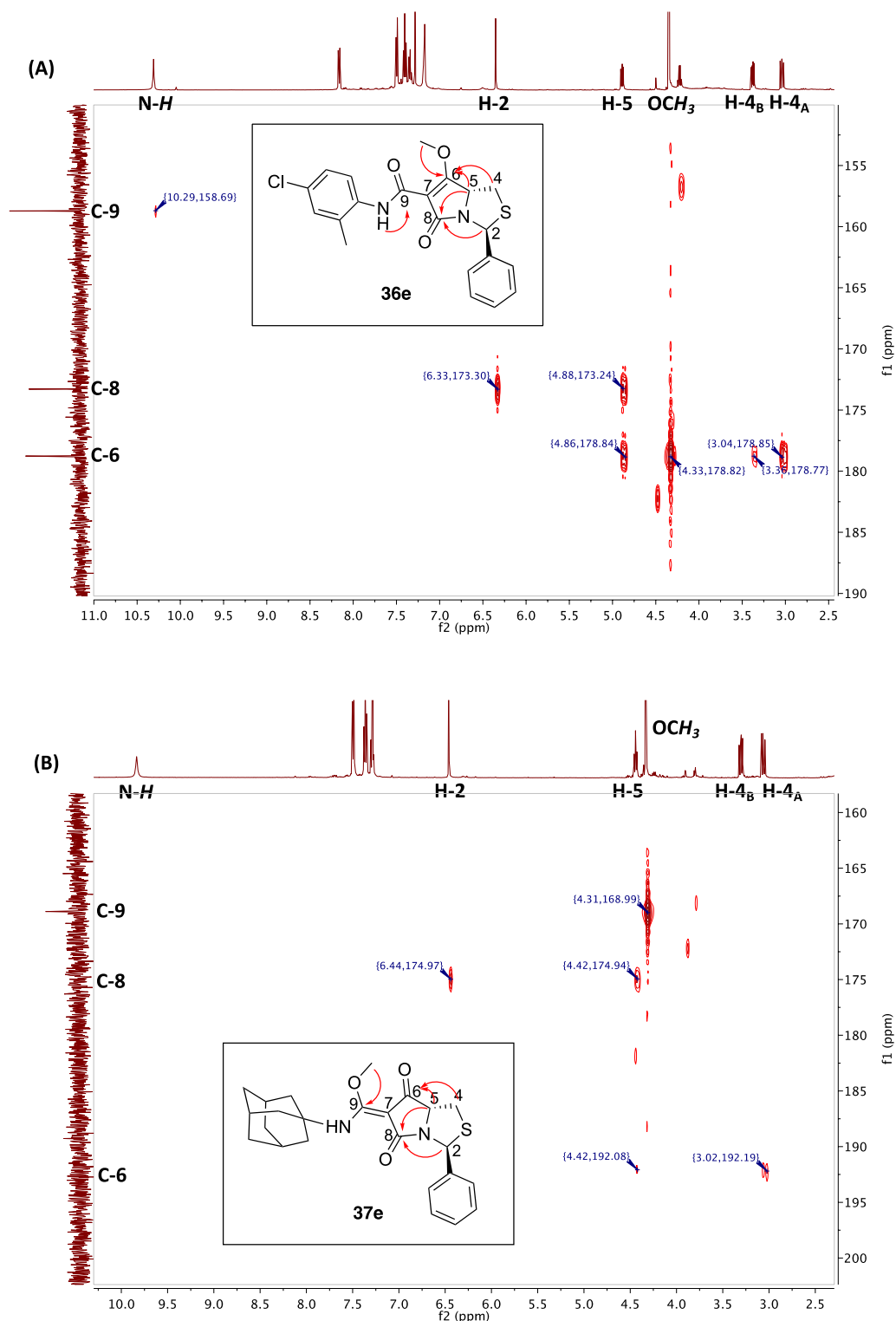
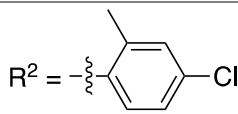
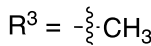
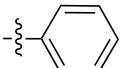
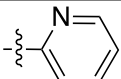
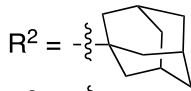
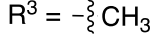
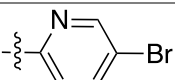
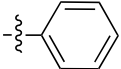
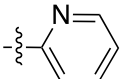
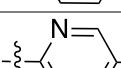
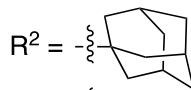
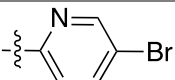
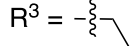
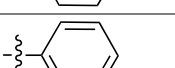
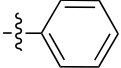
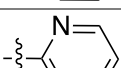
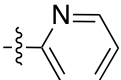
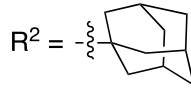
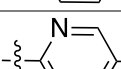
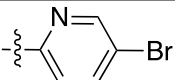
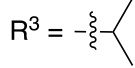
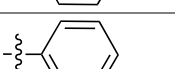
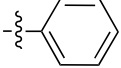
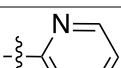
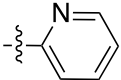
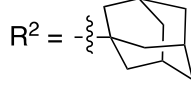
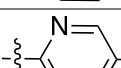
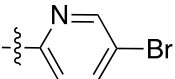
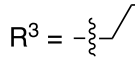
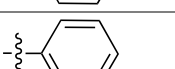
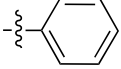
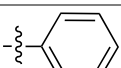
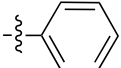
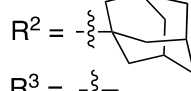
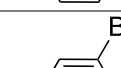
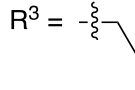
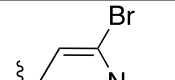
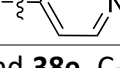


Figure 3.7 HMBC correlations of C-6 *O*-alkylated **36e** and C-9 *O*-alkylated **37e**.

Table 3.3 C-6, C-7, C-8, C-9 chemical shifts (CDCl₃) for the alkylated tetramates.

	R ² = / R ³ =	R ¹ =	δ (ppm)				Site of alkylation
			C-6	C-7	C-8	C-9	
36e	R ² =  R ³ = 		178.8	102.4	173.3	158.7	C-6 O
37a			193.6	87.0	176.6	169.1	C-9 O
37c	R ² =  R ³ = 		192.4	86.9	174.8	169.1	C-9 O
37e			192.2	87.1	174.9	169.0	C-9 O
38a-1			196.2	87.4	180.7	167.9	C-9 O
38a-2			179.2	96.9	177.2	162.1	C-6 O
38c-1	R ² = 		196.1	87.3	180.6	167.9	C-9 O
38c-2	R ³ = 		-	97.0	175.0	161.0	C-6 O
38e-1			196.1	87.5	181.1	167.9	C-9 O
38e-2			-	97.3	175.1	160.5	C-6 O
39a-1			192.8	87.6	176.2	166.8	C-9 O
39a-2	R ² = 		175.5	102.8	174.4	160.5	C-6 O
39c-1			192.7	87.6	176.2	166.8	C-9 O
39c-2	R ³ = 		176.6	102.8	174.5	160.0	C-6 O
39e-1			192.4	87.7	175.6	166.7	C-9 O
39e-2			175.6	103.1	174.3	160.2	C-6 O
40a-1			191.2	87.4	173.1	167.6	C-9 O
40a-2	R ² = 		176.7	103.6	173.7	159.9	C-6 O
40c-1			192.5	87.4	173.2	167.6	C-9 O
40c-2	R ³ = 		175.0	100.2	169.5	159.6	C-6 O
40e-1			192.4	87.6	175.1	167.6	C-9 O
40e-2			176.0	103.8	173.7	159.9	C-6 O
7R-41e			201.8	68.0	170.9	162.9	C-7 C
7S-41e	R ² = 		200.1	69.1	171.7	160.3	C-7 C
41b	R ³ = 		201.4	69.9	172.1	163.1	C-7 C
			199.6	70.0	172.2	160.1	C-7 C

[–] In ¹³C NMR spectra of **38a** and **38e**, C-8 chemical shift was not observed for the minor isomer, due to the intrinsic difficulty to detect C-8 as well as its relatively low proportion in the isolated mixture (17% and 10% respectively).

The different positions of alkylation brought out by a point change in the C-9 amide pendants indicated a potential for control of the alkylation site, where the electronic nature of the amide pendants could affect the relative stability of resonance structures of reaction intermediate **42** (Scheme 3.6, page 80, *vide supra*). The relative proportion of two regioisomers were also strongly influenced by the steric bulkiness of the incoming alcohol, with the ratio between them roughly conserved within a series of the same C-9 amide pendant and the same alcohol (i.e. same R² and R³ groups) (Table 3.2, page 83, *vide supra*).

By comparing the set of carbon chemical shifts to that observed in the model compounds **36e** and **37e**, structures of tetramate analogues synthesised under the Mitsunobu conditions were assigned (Table 3.3, page 86, *vide supra*). C-6, C-8 and C-9 chemical shifts were consistently more downfield in the C-9 *O*-alkylated isomers than in the C-6 *O*-alkylated ones, with a slight discrepancy in the case of **40a** where C-8 chemical shift in the C-9 *O*-alkylated isomer **40a-1** was more upfield than that of the C-6 *O*-alkylated **40a-2** but only with a difference of 0.6 ppm. The assignment of C-8 chemical shifts in **40a** was unequivocal because the two isomers were separated by column chromatography and analysed individually. The trend was reversed in C-7 chemical shifts, with those of the C-9 *O*-alkylated series being invariably more upfield. The set of chemical shifts was also distinct from that observed in the C-8 *O*-alkylated tetramate ketone analogues previously synthesised in the Moloney group (Scheme 3.7, page 81, *vide supra*).¹¹² The assignment of alkylation sites was further confirmed by HMBC correlation analysis of selected tetramate analogues (Figure 3.8).

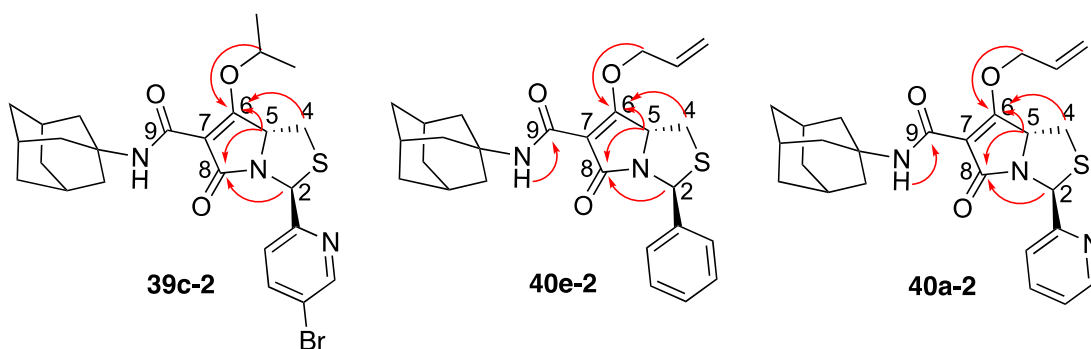


Figure 3.8 Examples of HMBC correlations confirming the assigned site of alkylation.

Combining the characteristic ^1H and ^{13}C chemical shift data (Table 3.2, page 83 and Table 3.3, page 86, *vide supra*), tetramate analogues **36-40** demonstrated consistent trends in the H-2 and H-5 chemical shifts, with H-2 being invariably more downfield in C-9 *O*-alkylated isomers than in C-6 *O*-alkylated isomers. This trend was reversed for H-5 chemical shifts.

Interestingly in the case of **41e** and **41b**, the sets of proton and carbon chemical shifts did not conform to either of the two sets previously observed in C-9 or C-6 *O*-alkylated series (Table 3.2, page 83 and Table 3.3, page 86, *vide supra*), suggesting that they were alkylated at a different position other than C-9 or C-6. The more downfield C-6 chemical shifts at around 200 ppm also indicated the presence of a ketone functional group instead of a conjugated enol group. HMBC correlation data of **41e** indicated that the C-6 carbon was correlated with H-4 and H-5 protons, C-8 correlated with H-2, H-5 and weakly with H-4 protons, C-9 correlated with N-H proton (Figure 3.9). The HMBC correlations between the protons on the alkylated side chain and C-7, but not with any carbonyl carbons, supported a tetramate structure with C-7 alkylation (Figure 3.9). The chemical shifts corresponding to protons on the alkylated side chain, consistent with vinyl protons

instead of allylic protons, further confirmed the structure as drawn instead of the alternative **41e'** (Figure 3.10). The HMBC correlations to C-7 were also stronger with the methyl protons (2-bond correlation) than the two vinyl protons (3-bond correlation). The C-7 alkylated tetramate carboxamide **41e** was synthesised as a 1 : 5 mixture of 7*R*/*S* diastereomer with a *trans*-relationship between H-2 and H-5. The major 7*S* diastereomer was isolated in 15% yield and its relative stereochemistry confirmed by NOE analysis (Figure 3.11). Compound **41b** was however a 1 : 1 7*R*/*S* mixture of diastereomers.

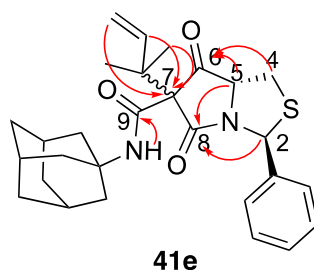


Figure 3.9 HMBC correlation analysis of alkylated tetramate carboxamide **41e**.

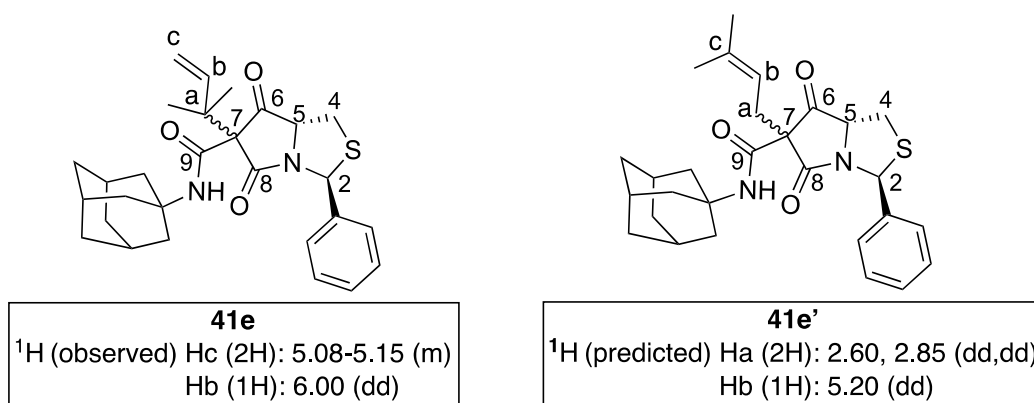


Figure 3.10 Chemical shifts of alkyl chain protons of tetramate carboxamide **41e**. The predicted ¹H chemical shifts were calculated by ChewDraw Professional 19.1.1.32.

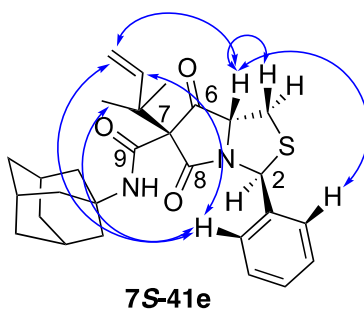
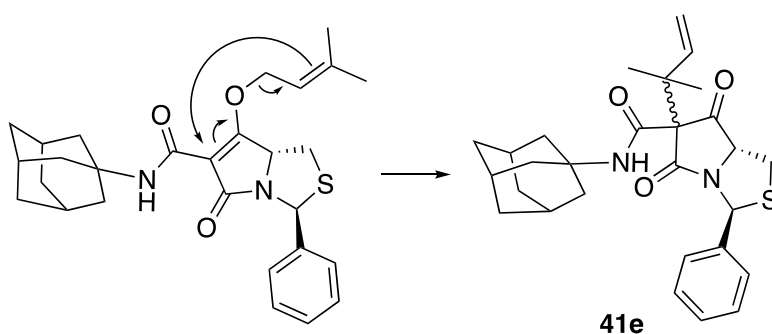
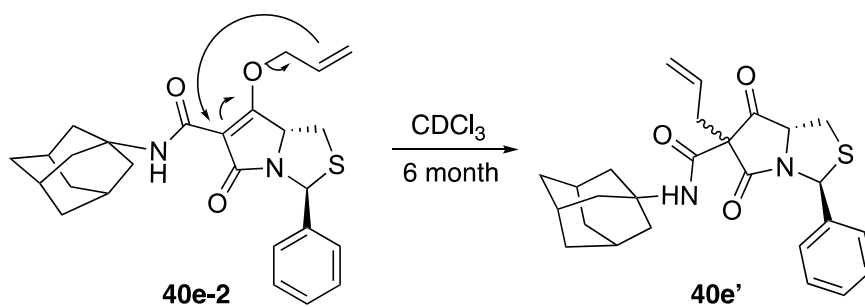


Figure 3.11 NOE analysis of alkylated tetramate carboxamide **7S-41e**.

Based on the structure of **41e**, a mechanism of C-7 alkylation of the tetramate analogue under Mitsunobu conditions with prenyl alcohol was suggested where O-alkylation happened first, and this was followed by a migration of the prenyl side chain (Scheme 3.9). A similar migration was possible with the allyl derivative (Scheme 3.10). This was indeed observed when **40e-2** was kept in CDCl₃ solution over an extended duration, a second set of peaks emerged and increased in concentration (Figure 3.12). The chemical shifts of the resulting **40e'** were comparable to those observed in **41e**, suggesting a similar C7 alkylated product (Table 3.4). It appeared that **40e'** was a single diastereomer at C-7.



Scheme 3.9 Proposed mechanism for the observation of C-7 alkylated carboxamide **41e**.



Scheme 3.10 Proposed mechanism for allyl migration to C-7 alkylated **40e'**.

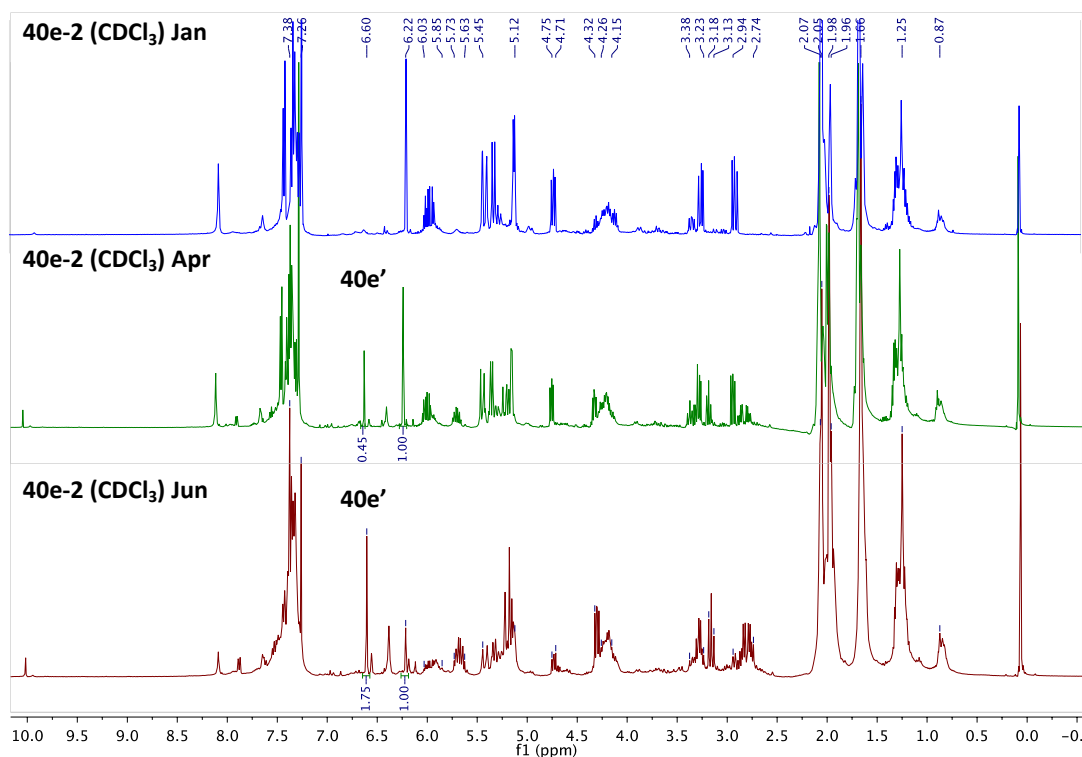


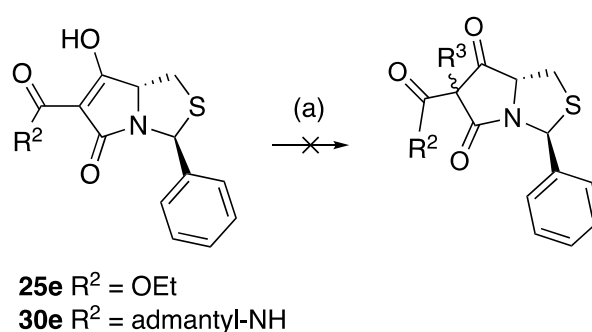
Figure 3.12 ¹H NMR spectra (CDCl₃, 400 MHz) of **40e-2** over time.

Table 3.4 Comparing characteristic chemical shifts (CDCl₃) between **41e** and **40e'**.

	R ² = / R ³ =	R ¹ =	δ (ppm)					
			H-2	H-5	C-6	C-7	C-8	C-9
7R-41e	R ² =		6.58	4.54 (m)	200.5	68.6	170.4	160.8
7S-41e	R ³ =		6.55	4.33 (dd)	200.1	69.1	171.6	160.3
40e'	R ² = R ³ =		6.60	4.30 (dd)	200.9	69.7	171.6	162.6

3.3.2 Attempted synthesis of tetramate carboxamides with a quaternary C-7 centre

In view of the interesting C-7 alkylation observed under the Mitsunobu conditions, synthesis towards similar tetramate carboxamides with a possibility of diversifying C-7 alkylated R³ group was attempted. Unfortunately, C-7 alkylation was not successful for either tetramate ester or carboxamide under the conditions of NaH/Mel, with degradation of the starting materials (Scheme 3.11).

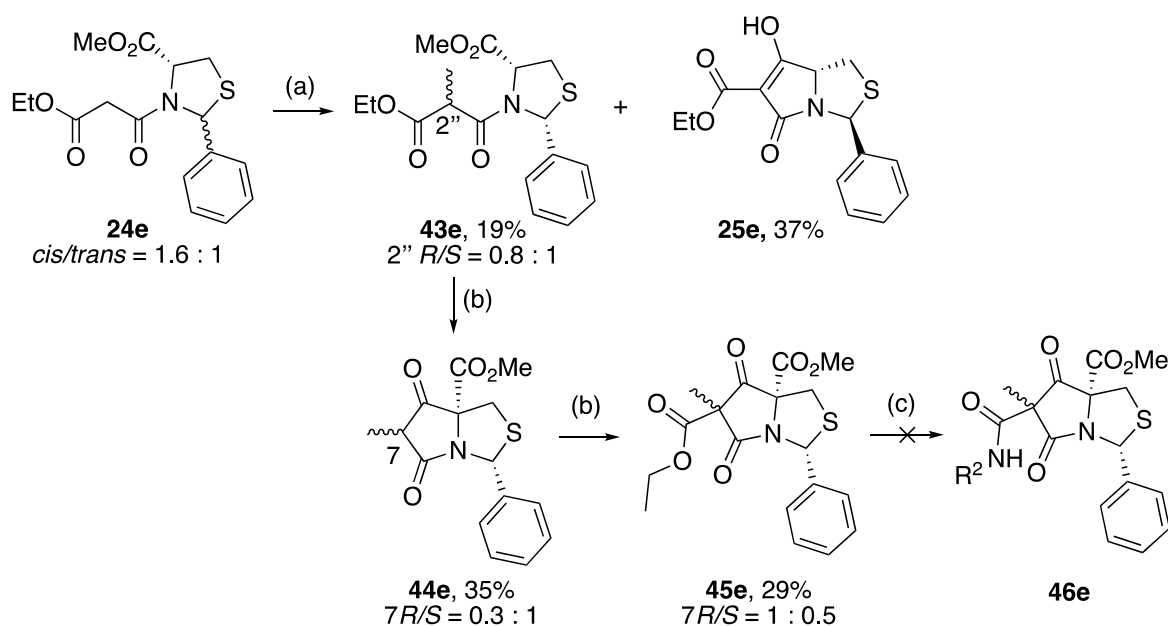


Scheme 3.11 Attempted C-7 alkylation of tetramate analogues. *Reagents and conditions:*

(a) NaH, MeI, anhydrous THF, 0 °C to r.t., o.n.

C-alkylation of the side chain of the *N*-acylthiazolidine was however possible under the same conditions, although with simultaneous Dieckmann cyclisation under the basic conditions of NaH producing tetramate ester **25e** as a major product in 37% yield (Scheme 3.12). The C-alkylated *N*-acylthiazolidine **43e** as a minor product was isolated in 19% yield and taken for a separate Dieckmann cyclisation with KO^tBu in THF under reflux. The subtle change in the steric bulkiness of the side chain by an additional methyl group altered the direction of cyclisation, leading to C-5 methyl ester tetramate analogue **44e** in 35% yield as a 0.3 : 1 mixture of diastereomers (*7R/S*) with a *cis*-relationship between H-2 and H-5. This tetramate analogue **44e** was reacted with ethyl chloroformate under the

basic conditions of DMAP to create a C-7 quaternary centre in 29% yield. However, both ester groups in the resultant tetramate analogue **45e** was inaccessible under the transamidation conditions, with 50% recovery of the starting material. Further investigation is needed to devise a synthetic pathway to access tetramate carboxamides with a quaternary C-7 centre.



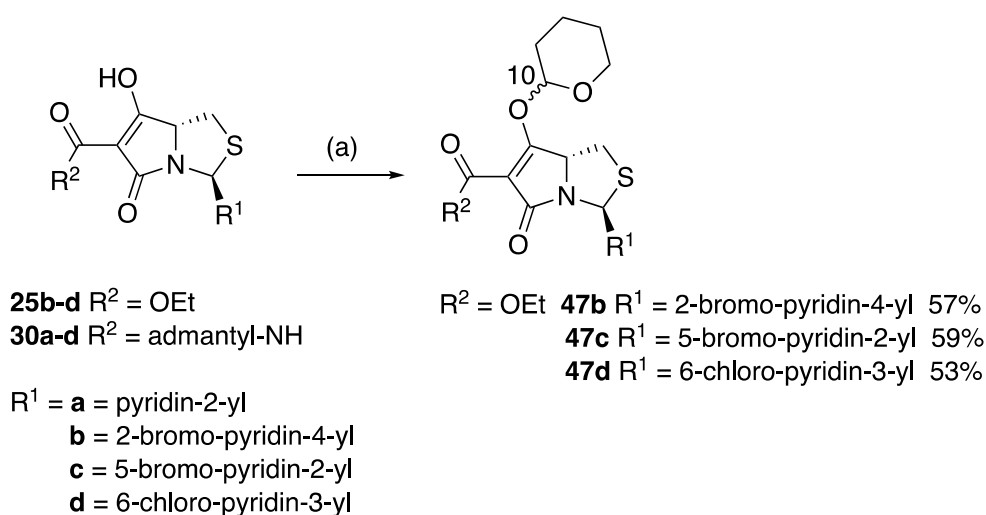
Scheme 3.12 Attempted synthesis of tetramate analogues of a C-7 quaternary centre.

Reagents and conditions: (a) NaH, MeI, anhydrous THF, 0 °C to r.t., o.n.; (b) KO^tBu, anhydrous THF, reflux, 4 h; (c) R²NH₂ (R₂ = 1-adamantyl), THF/toluene, reflux, o.n.

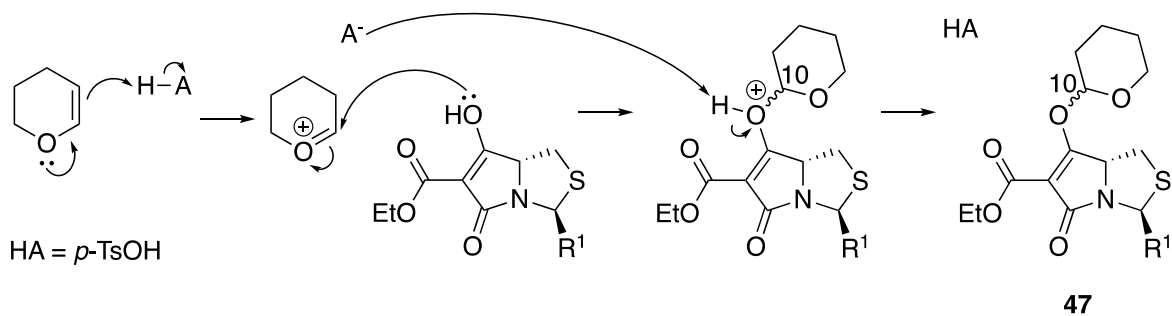
3.3.3 Towards the synthesis of THP-protected tetramate carboxamides

Relying on the reactivity of the C-6 hydroxyl group, another set of tetramate carboxamides was proposed with incorporation of a THP protecting group, which bears some structural similarity to a sugar derivative (Scheme 3.13). Under the reported conditions of THP protection,¹⁶¹ it appeared that tetramate esters were reactive to

further *O*-alkylation in moderate yields (53 - 59%), but carboxamides failed to react, with recovery of the starting materials. This was consistent with observation of the relative reactivity between tetramate esters and carboxamides under the Mitsunobu conditions. The THP-protected tetramate esters were synthesised as a mixture of 1 : 1 diastereomers at C-10 according to the proposed mechanism (Scheme 3.14).¹⁶¹ The characteristic and consistent chemical shifts of the tetramate analogues **47b-d** were summarised in Table 3.5.

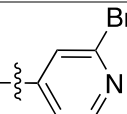
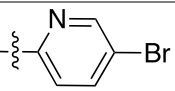
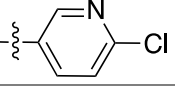


Scheme 3.13 THP protection of tetramate esters. *Reagents and conditions:* (a) *p*-TsOH monohydrate, 3,4-dihydropyran, DCM, r.t., o.n.



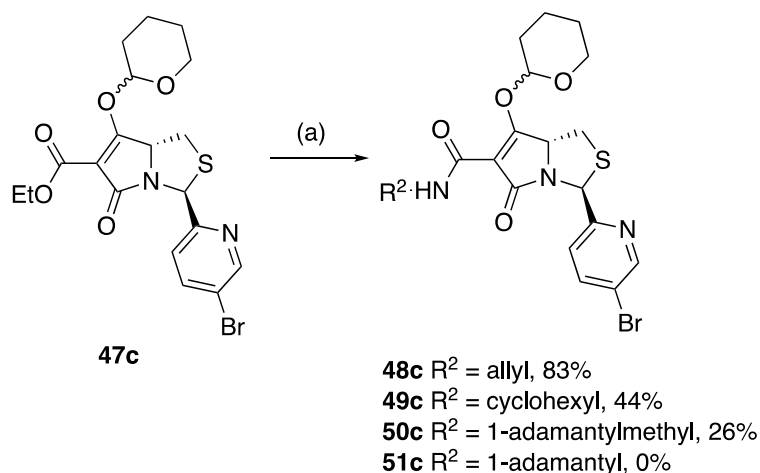
Scheme 3.14 Proposed mechanisms of THP protection in tetramate esters.

Table 3.5 Yield, H-2, C-6/7/8/9 chemical shifts (CDCl₃) for THP-protected tetramate esters.

	R ¹ =	δ (ppm)					Ratio	% Yield ^a
		H-2	C-6	C-7	C-8	C-9		
47b		6.50	200.8	80.5	167.6	163.3	1 : 1	57
		6.44	200.3	79.3	166.7	163.2		
47c		6.58	201.7	80.4	167.8	163.5	1 : 1	59
		6.52	201.2	79.3	167.2	163.4		
47d		6.58	201.2	80.3	167.8	163.4	1 : 1	53
		6.52	200.7	79.2	167.3	163.3		

[a] Isolated yield from flash column chromatography.

As direct THP protection of tetramate carboxamides failed, transamidation of a THP-protected tetramate ester **47c** was attempted to synthesis the desired THP-protected tetramate carboxamides (Scheme 3.15). However, the aminolysis reactions seemed to be limited by the steric bulkiness of the primary amines, with drastic decrease in yield when a bulky admantylamine was used instead of allylamine, despite the addition of L-proline as a catalyst.¹⁶² The characteristic and consistent H-2 chemical shifts of the tetramate analogues **48-50c** were summarised in Table 3.6. It should however be noted that such aminolysis procedures had worked exceptionally well in non-protected tetramate esters, with reported yield of 30 - 92% depending on the amines.^{48,110,163} Successful aminolysis on the same tetramate system but with C-6 free hydroxyl group was also demonstrated (Table 2.4, page 57, *vide supra*).



Scheme 3.15 Transamidation reactions of a THP-protected tetramate ester **47c**. *Reagents and conditions:* (a) R²NH₂, THF/toluene, reflux, 24 - 48 h.

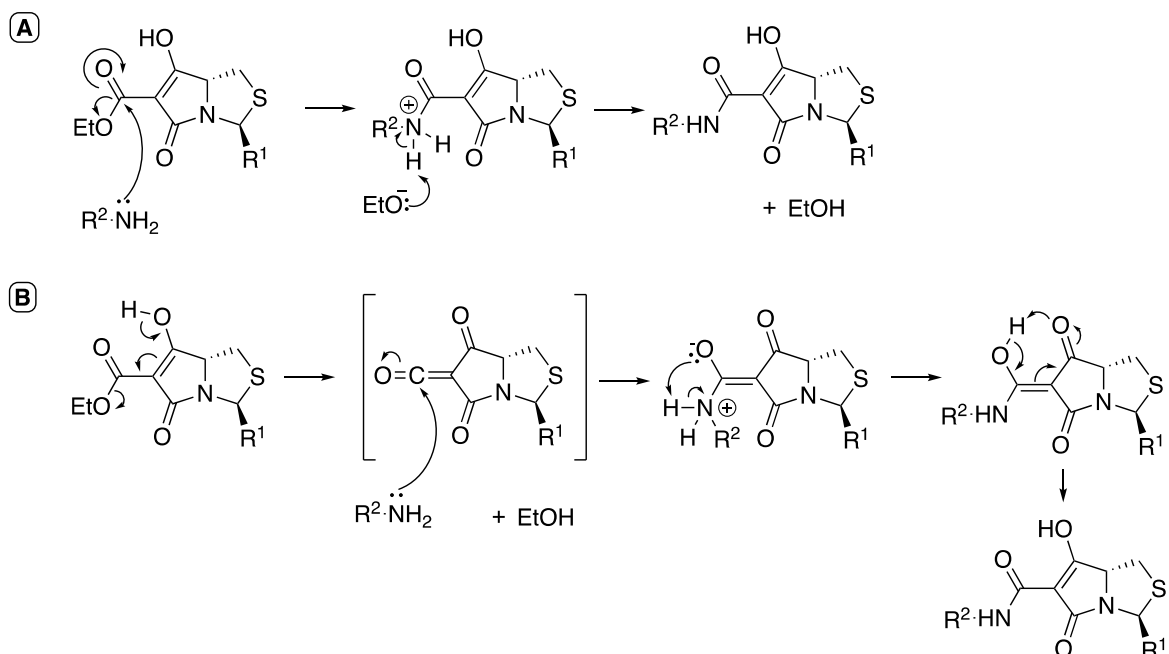
Table 3.6 Yield, H-2, C-6/7/8/9 chemical shifts (CDCl₃) for THP-protected carboxamides.

	R ² =	R ¹ =	δ (ppm)					Ratio	% Yield
			H-2	C-6	C-7	C-8	C-9		
48c			6.13	169.4	78.4	165.9	165.7	0.5	83
			6.25	168.9	78.4	167.1	165.9	1	
49c			6.13					0.6	-
			6.29					1	44
50c			6.02			-		0.6	26
			6.14					0.8	
			6.28					1	

[–] ¹³C NMR data not available for **49c** and **50c**.

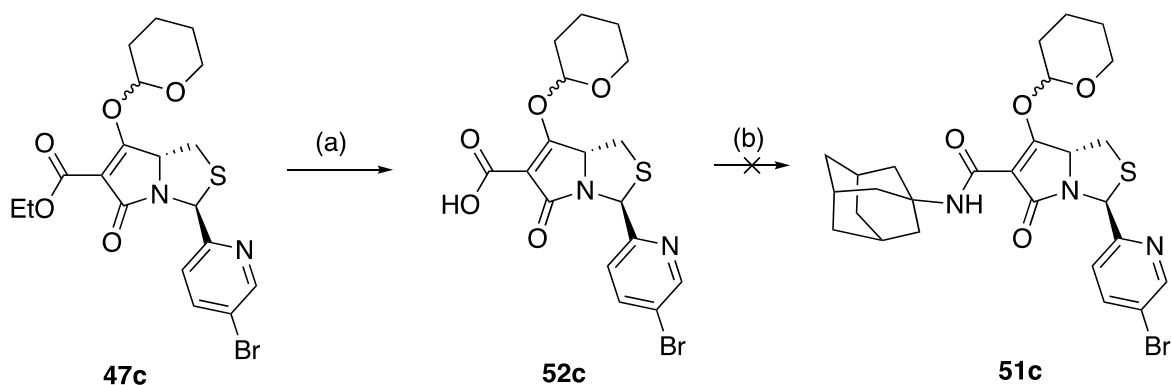
The difficulty encountered in the transamidation reactions with C-6 enol protection as compared to a C-6 free hydroxyl group suggested an alternative for the mechanism of aminolysis in the tetramate systems (Scheme 3.16). Besides the pathway (A) from direct nucleophilic attack of the amino group to the ester carbonyl carbon, a second pathway (B) was possible with activation from the C-6 hydroxyl group, leading to a reactive ketene intermediate (Scheme 3.16). In aminolysis reactions of C-6 enol protected tetramate

esters, pathway (B) was no longer possible while pathway (A) was affected by the bulkiness of the nearby THP group and the incoming amine.



Scheme 3.16 Proposed mechanisms of aminolysis in tetramate esters.

Another synthetic route to THP-protected tetramate carboxamide **51c** with hydrolysis of the corresponding THP-protected tetramate ester **47c** was explored (Scheme 3.17). While the desired THP-protected tetramate analogue with C-7 carboxylic acid **52c** was detected by mass spectrometry, its isolation was difficult with significant polarity of the product. This was in contrast with previous attempts of hydrolysis of tetramate esters with a free C-6 hydroxyl group, where decarboxylation and dimerisation occurred.^{104,156} The crude product after work-up was used directly for subsequent coupling reactions, which were also unsuccessful in producing the desired tetramate carboxamide **51c**.



Scheme 3.17 Attempted synthesis of THP-protected tetramate carboxamide **51c** with hydrolysis of the corresponding tetramate ester **47c**. *Reagents and conditions:* (a) LiOH monohydrate, THF/H₂O (1:1), r.t., o.n.; (b) 1-adamantylamine, DCC, DMAP, DCM, 0 °C to r.t., 18 h; or 1-adamantylamine, T₃P, Et₃N, DMF, 0 °C to r.t., 18 h.

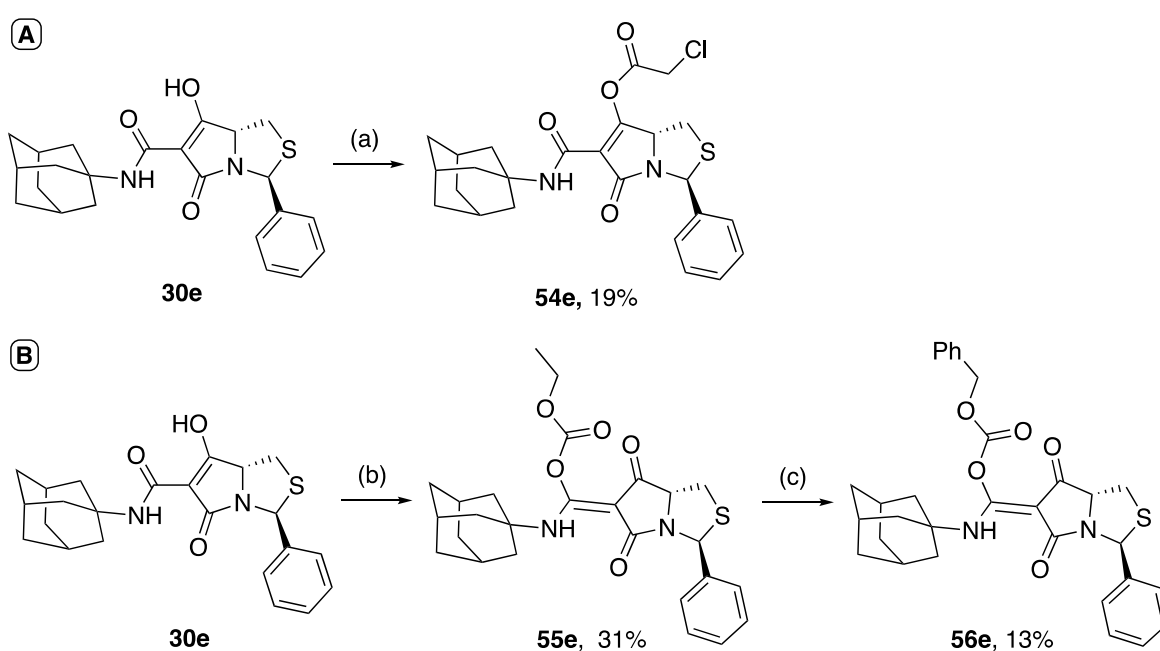
The synthesised THP-protected tetramate carboxamide **48c** was used as a model compound to investigate its potential to release the parent tetramate carboxamide **53c** under specific reaction conditions (Table 3.7). However, it failed to deprotect under all attempted conditions with recovery of the starting material, indicating an unexpected structural stability of this tetramate system, probably due to the non-basicity of the C-6 enol oxygen.

Table 3.7 Attempted THP deprotection for tetramate carboxamide **48c**.

Conditions ¹⁶¹	Remarks
1 <i>p</i> -TsOH in MeOH, r.t. or reflux, o.n.	Recovery of starting THP-protected tetramate carboxamide 48c ;
2 TFA in DCM or neat TFA, r.t., o.n.	No parent tetramate carboxamide 53c detected by mass spectrometry.
3 PPTS in ethanol, 55°C, o.n.	
4 4M HCl, dioxane/DCM 1 : 1, r.t., o.n.	

3.3.4 Synthesis of tetramate analogues with linker systems

Given the difficulty encountered in the synthesis of THP-protected tetramate carboxamides and the failure to deprotect subsequently, further attempts were made to functionalise the C-6 hydroxyl group with short chain linkers with in-built structural instability and to investigate further the possibility for sugar derivative incorporation in the future (Scheme 3.18).



Scheme 3.18 Synthesis of tetramate carboxamides with linker systems. *Reagents and conditions:* (a) chloroacetyl chloride, anhydrous Et₃N, DCM, 0 °C to r.t., o.n.; (b) ethyl chloroformate, anhydrous Et₃N, DCM, 0 °C to r.t., o.n.; (c) benzyl alcohol, catalytic *p*-TsOH monohydrate, toluene, reflux, 48 h.

Reaction of tetramate carboxamide **30e** with chloroacetyl chloride under the basic conditions of Et₃N produced **54e** in 19% yield (Scheme 3.18 A). Reaction with the more reactive counterpart, bromoacetyl bromide failed with degradation of the starting

material, probability due to the nucleophilic nature of the thioether present in the tetramate system. Addition of a catalytic amount of DMAP did not improve the yield of the reaction. ^1H NMR spectroscopic analysis of the relevant proton and carbon chemical shifts indicated that the product **54e** as an exclusively C-6 *O*-alkylated tetramate analogue, with characteristic chemical shifts generally comparable to those C-6 *O*-alkylated tetramates **36-40** formed under the Mitsunobu conditions (Table 3.8; Table 3.2, page 83 and Table 3.3, page 86, *vide supra*). The assignment of carbonyl carbon chemical shifts was confirmed by HMBC correlations where C-6 correlated with H-4 and H-5, C-8 correlated with H-2 and H-5, and the additional carbonyl C-10 correlated to the protons on the alkyl side chain (Figure 3.13). C-6 of **54e** was more upfield than that in other C-6 *O*-alkylated tetramate enol ethers, which suggested that C-6 was attached directly to an ester group as opposed to the alternative enol ether analogue **54e'** (Figure 3.14). This was consistent with the prediction that the acid chloride was more reactive than the alkyl chloride in chloroacetyl chloride. Although the presence of a halogen on the linker allowed functionalisation by nucleophilic substitutions, the low yield of the reaction discouraged further investigations.

Table 3.8 Characteristic ^1H , ^{13}C chemical shifts of tetramate analogues **54-56e**.

	$\text{R}^3 =$	$\text{R}^1 =$	δ (ppm)					% Yield	
			H-2	H-5	C-6	C-7	C-8		C-9
54e			6.22	5.01 (dd)	168.0	104.2	169.4	160.3	19
55e			6.43	4.40 (t)	192.4	87.5	175.1	167.8	30 ^a
56e			6.47	4.46 (t)			-		13

[a] Average isolated yield calculated from multiple attempts. [-] ^{13}C NMR data not available for **56e**.

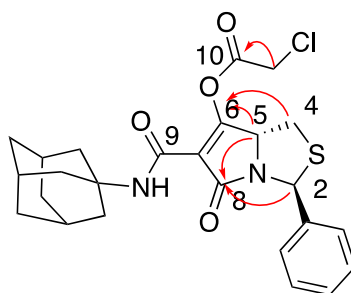


Figure 3.13 HMBC correlation analysis of **54e** for the assignment of carbonyl carbon chemical shifts. C-6 correlated with H-4 and H-5, C-8 correlated with H-2 and H-5, C-10 (164.8 ppm) correlated with C-11 protons on the side chain and carbonyl carbon C-9 was assigned last by way of elimination.

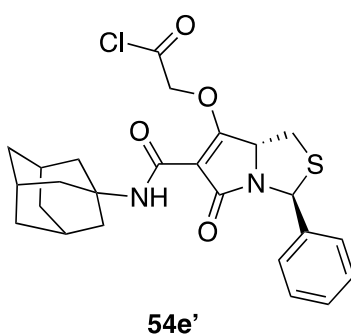


Figure 3.14 Alternative enol ether analogue **54e'**, which was not observed.

Reaction of the same tetramate carboxamide **30e** with ethyl chloroformate under the basic conditions produced tetramate analogue **55e** with a carbonate ester linker (Scheme 3.18 B, page 99, *vide supra*), which was intrinsically unstable by hydrolysis under physiological conditions. NMR analysis indicated that **55e** was an exclusively C-9 *O*-alkylated tetramate product (Table 3.8, page 100 and Table 3.3, page 86, *vide supra*). The structural assignment was further supported by HMBC correlation analysis (Figure 3.15). The difference in C-6 and C-8 chemical shifts to the ethyl ether counterpart **38e-1**, as well as the presence of an additional carbonyl carbon peak at 163.8 ppm, supported the

structure of **55e** as drawn. However, as the carbonate ester in **55e** was intrinsically unstable to hydrolysis, minor $[M+H]^+$ and $[M+K]^+$ peaks corresponding to **38e** were detected by mass spectrometry. This mass ion M could arise from **55e**-CO₂, supporting the instability of the structure.

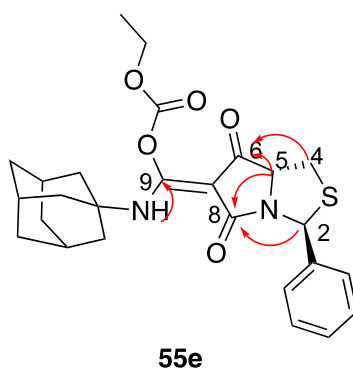
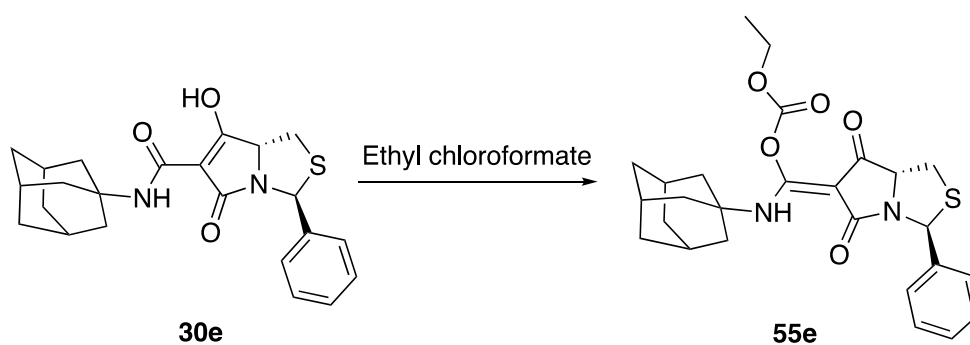


Figure 3.15 HMBC correlation analysis of **55e**. C-6 correlated with H-4 and H-5, C-8 correlated with H-2 and H-5, C-9 correlated with N-H.

Reaction optimisation for the production of **55e** was carried out with changes in solvent, temperature and base, unfortunately with no significant improvement in yield could be achieved (Table 3.9). Tetramate analogue **56e** was produced from **55e** with a transesterification reaction in 13% yield (Scheme 3.18 B, page 99, *vide supra*); the characteristic H-2 and H-5 proton chemical shifts suggested a similar C-9 *O*-alkylated structure preserved from **55e** (Table 3.8, page 100, *vide supra*). However, the harsh conditions of transesterification also turned **55e** to the ethyl ether **38e-1**, isolated in 40% yield.

Table 3.9 Reaction optimisation for the production of **55e**.

Reaction conditions		% Yield ^a
1	Et ₃ N, DCM, 0 °C to r.t., o.n.	31
2	Et ₃ N, DCM, reflux (40 °C), o.n.	33
3	Et ₃ N, catalytic DMAP, DCM, reflux, o.n.	31
4	Et ₃ N, toluene, 90 °C, o.n.	36
5	DIPEA, toluene, 90 °C, o.n.	30
6	Et ₃ N, toluene, reflux at 110 °C, o.n.	21

[a] Isolated yield from flash column chromatography.

3.4 Metal chelating properties of the synthesised tetramates

In contrast to the previously synthesised tetramate carboxamides **27-30(a-d)** with strong metal chelation abilities (Figure 2.16, page 63, *vide supra*), the tetramate analogue **35** with a C-9 C=N bond in replacement with a carbonyl group was found to be only weakly metal-binding, as indicated by the slight broadening of peaks in its ¹H NMR spectrum after purification with flash silica column chromatography (Figure 3.16 A). It was washed with 5% aqueous citric acid solution to render metal-free material (Figure 3.16 B). Tetramate carboxamides **36-41**, **48-50**, **54-56** were all non-metal chelating as suggested by the sharp and resolved ¹H NMR spectra directly after column chromatography (Figure 3.16 C-F). The stark change in the metal chelation property of tetramate analogues with modifications at C-9 carbonyl and C-6 enol functionalities again highlighted the structural dependency of such ability, supporting the direct involvement of C-9 carbonyl O and C-6 deprotonated O in metal complexation.

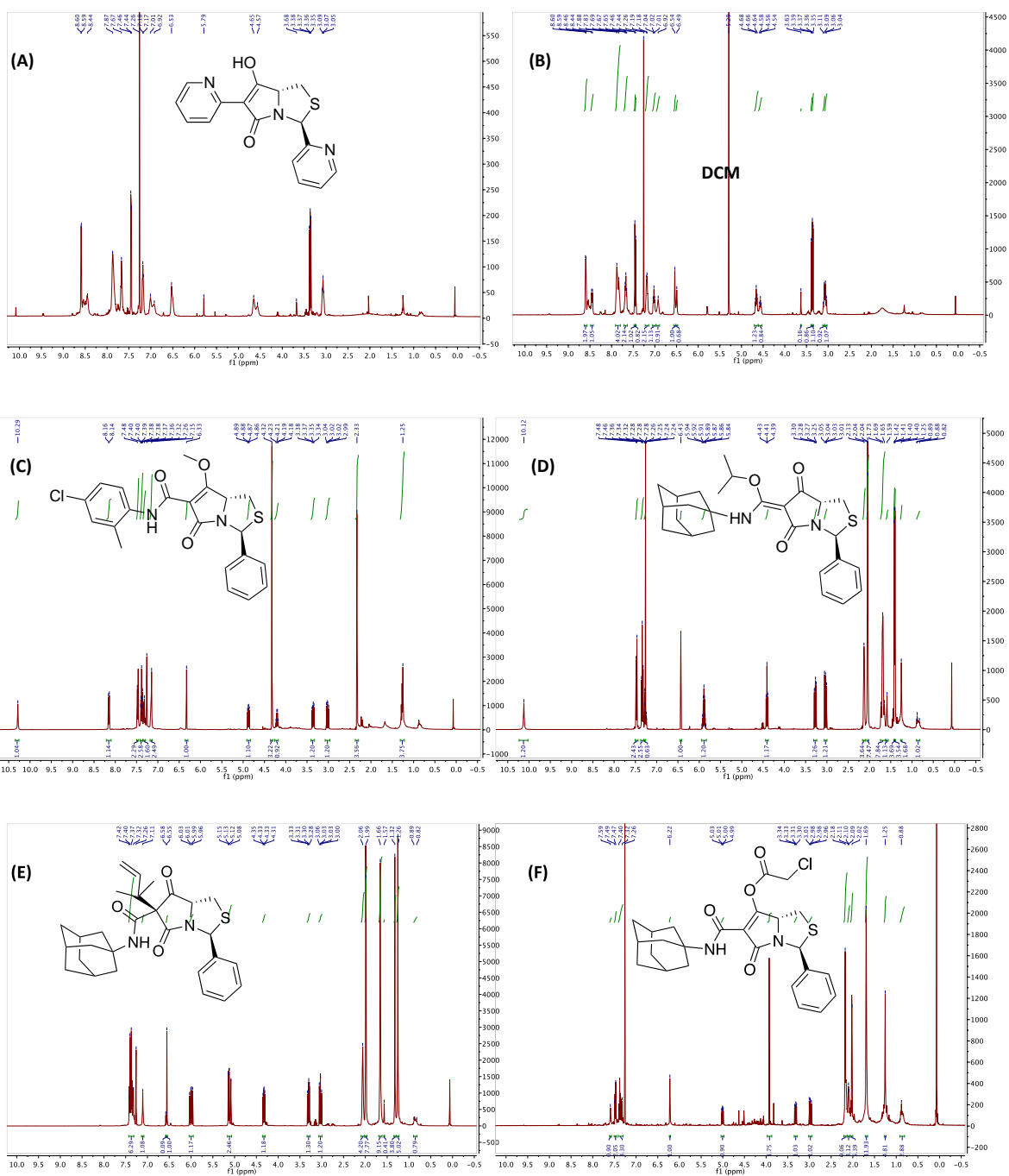


Figure 3.16 ^1H NMR (CDCl_3 , 400 MHz) spectra indicating metal-chelation abilities of the synthesised tetramate analogues. (A) **35**, with a C-9 C=N bond, post-column, before acidic wash; (B) **35** post-column, after 5% citric acid wash; (C) C-6 O-alkylated **36e** post-column; (D) C-9 O-alkylated **39e** post-column; (E) C-7 Alkylated **7S-41e** post-column; (F) Tetramate analogue with a short chain linker **54e** post-column.

3.5 Biological evaluation

The tetramate derivatives **32** and **35** with alterations at C-9 carbonyl group and tetramate carboxamides **36-41**, **48-50**, **54-56** were tested for their biological activities against Gram-positive MRSA (some also tested against Gram-positive *Streptomyces*) and Gram-negative *E. coli* by MIC assays. No activity was seen against the Gram-negative *E. coli*. Neither **32** nor **35** showed any activity. The metal chelation study indicated that **32**, with a C-9 C=C bond was not metal-chelating and **35** with a C-9 C=N bond was very weakly metal binding (Figure 3.16 A-B, page 104, *vide supra*), suggesting that C-9 carbonyl was essential for metal chelation, which was also important to the antibacterial activity of the tetramate derivatives. C-9 *O*-alkylated tetramate carboxamides **40a-1**, **55e** and **56e** were found to be weakly active against Gram-positive MRSA (and *Streptomyces*) (Table 3.10). The instability of these tetramate systems suggested the likelihood of releasing the active parent tetramate carboxamides under physiological conditions, correlating to the exhibited antibacterial activities. All THP-protected tetramate carboxamides were inactive, probably due to the inability to deprotect under physiological conditions. Interestingly, C-7 alkylated tetramate carboxamides **41e** and **41b** demonstrated promising antibacterial activities against Gram-positive MRSA (and *Streptomyces*). These tetramate derivatives were non-metal chelating as indicated by the sharp and resolved post column ¹H NMR spectra (Figure 3.16 C-F, page 104, *vide supra*), suggesting that they acted via a mechanism independent from metal binding, different from the previously synthesised tetramate analogues. The activity of **41e** was again mitigated in the presence of blood due to its high lipophilicity and hence extensive binding to plasma protein HSA; the extent of plasma protein binding could be reduced with the more polar analogue **41b**, although with a less potent biological activity, thus warranting further optimisation. It was also

important to note the effect of C-7 configuration on antibacterial activity, with 7S diastereomer appeared to be more active than 7R diastereomer. As the relative proportion of 7S diastereomer increased by 7%, there was a two-fold decrease in the MIC value, suggesting that the 1 : 2 7R/S-**41e** mixture was twice as active as the 1 : 1.5 mixture. The sample of **41b** was however a 1:1 mixture of 7R/S diastereomers, thus rendering the MIC values not directly comparable between **41b** and **41e**. A more detailed analysis of structure-activity relationship is described in Chapter 5.

Table 3.10 Biologically active cysteine-derived tetramate analogues.

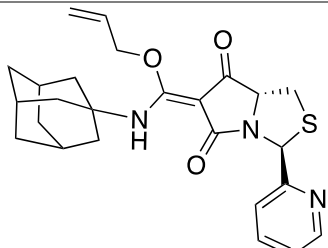
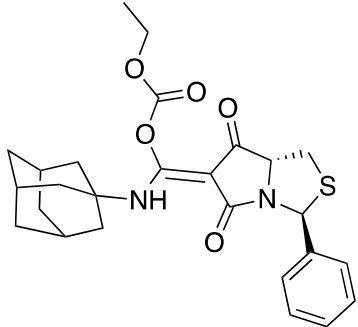
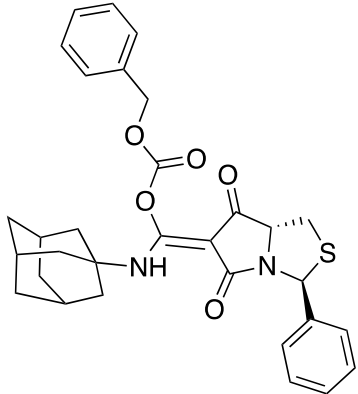
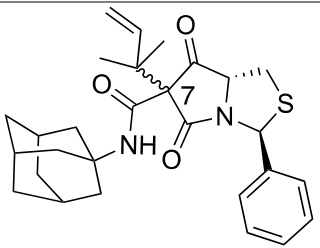
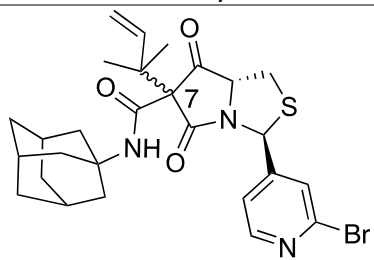
	Structure	MIC ($\mu\text{g/ml}$) against		MW	clogP ^a	PSA ^a /Å ²
		MRSA	<i>Streptomyces</i>			
40a-1		31.3	31.3	452	3.29	71.5
55e		31.3	Not tested	483	4.41	84.9
56e		15.6	Not tested	545	5.84	84.9

Table 3.10 Continued. Biologically active cysteine-derived tetramate analogues.

	Structure	MIC ($\mu\text{g/ml}$) against		MW	clogP ^a	PSA ^a /Å ²
		MRSA	<i>Streptomyces</i>			
41e				479	4.98	66.5
	1 : 2 7 <i>R/S</i>	1.95	Not tested			
	1 : 1.5 7 <i>R/S</i>	3.91	Not tested			
	7 <i>S</i> only ^b	7.81	3.95			
41b		15.6	Not tested	559	4.85	79.4
	1 : 1 7 <i>R/S</i>					

[a] Relevant chemical properties of the active compounds were calculated using MarvinSketch 20.3.0, 2020, ChemAxon; [b] The third sample of **41e** with exclusively 7*S* diastereomer was tested in a different batch than the other two samples, thus making the MIC values not directly comparable.

3.6 Suggestions for future work

As antibacterial activity was demonstrated by **41e** and **41b** with only one type of C-7 alkyl substituent ($C(CH_3)_2CH=CH_2$), but not the allyl counterpart **40e'**, the specificity of C-7 alkyl chain as well as its chiral configuration for the tetramate system would appear to be important. A stereoselective synthetic route to access C-7 alkylated tetramate library with a diversity of C-7 substituents (R^3) is needed to fully establish the structure-activity relationship (Figure 3.17).

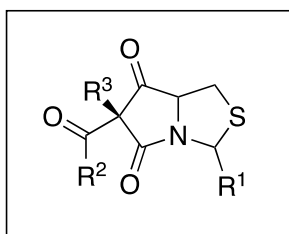
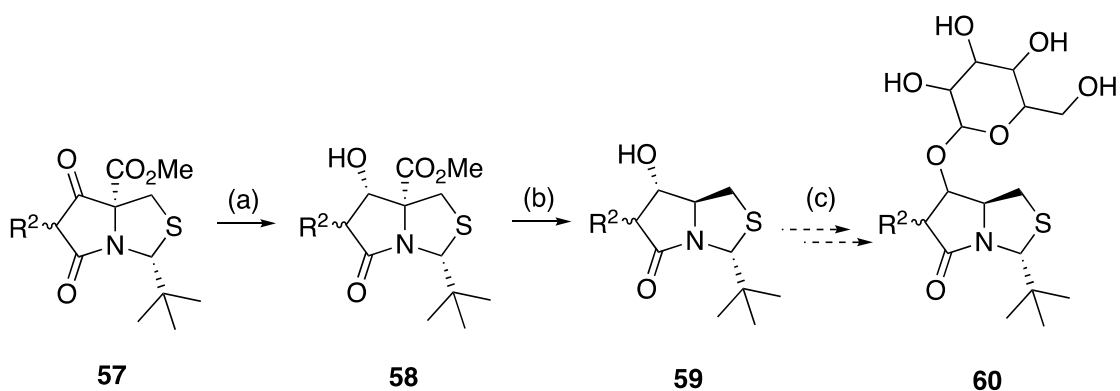


Figure 3.17 Proposed further target compounds with C-7 alkylation.

A second suggestion is to focus on the incorporation of sugar derivatives into the tetramate system at C-6 position. Given that the lone pair on the deprotonated C-6 enol was extensively delocalised in the tricarbonyl conjugated system under the Mitsunobu conditions, lowering its nucleophilic reactivity towards bulky alcohols, it is perhaps necessary to reduce the tetramate system to a structural template with a secondary hydroxyl group at C-6, which can be employed to couple with a sugar derivative (Scheme 3.19).



Scheme 3.19 Proposed route to access tetramate systems with sugar derivatives.

Reagents and conditions: (a) NaBH_4 , acetic acid, DCM, 0 °C to r.t., 2 h; (b) NaOH, THF/MeOH/ H_2O , reflux, 8 h, followed by MeCN, reflux, 5 h; (c) glycosylation conditions with a sugar derivative with anomeric hydroxyl group; followed by deprotection of hydroxyl groups on the sugar derivative.

The Moloney group has previously reported a stereoselective reduction of the tetramate core **57** to **58** with the resulting C-6 hydroxyl group exclusively in the *S* configuration.¹⁰⁸ Successful reduction was observed when $\text{R}^2 = \text{H}$ or alkyl chains, but not with the acylated counterparts.^{108,164} The presence of C-5 methyl ester was found to be crucial for the high stereoselectivity of the reaction.¹⁰⁸ When **58** was taken directly for a Mitsunobu reaction with a carboxylic acid, exclusive elimination occurred, suggesting the steric hindrance of the system towards coupling reactions.¹⁶⁵ Therefore, a decarboxylation reaction is planned in the synthetic route, according to previously reported conditions,¹¹² to reduce the steric bulkiness around the C-6 hydroxyl group for a subsequent coupling with a sugar derivative.¹⁶⁶ It should be noted that with reduction, the C-6 hydroxyl group is no longer acidic as in the enol functionality so that glycosylation should not be dependent on base catalysis.

3.7 Summary

This chapter described the synthesis of cysteine-derived tetramate analogues with functionalisation at C-9 carbonyl and C-6 enol groups. The aim of such modifications was to access the functional groups directing participating in metal complexation of the tetramate derivatives. The model tetramate derivative **35** with a C-9 C=N bond exhibited weak metal-chelating ability as indicated by the small extent of peak broadening in ¹H NMR spectra post column chromatography, which was apparently translated to a loss of antibacterial activity.

The main concept behind designing target compounds with C-6 hydroxyl functionalisation was to synthesis tetramate analogues as prodrugs that could be hydrolysed by bacterial enzymes to release the active parent tetramates. Alkylation under the Mitsunobu conditions for tetramate derivatives with an extended tricarbonyl conjugated system led to potential regioisomers alkylated at different positions, which were distinguished by HMBC correlation analysis and tabulation of carbonyl carbon chemical shifts. C-9 and C-6 *O*-alkylation were observed but not C-8 *O*-alkylation for tetramate carboxamides; interestingly, C-7 alkylation with allyl and prenyl derivatives was also observed. The C-7 alkylated tetramate derivatives **41e** and **41b** with apparently no metal chelating ability demonstrated promising antibacterial activity against MRSA, suggesting a different mode of action of tetramate analogues independent from metal binding. The synthetic route leading to the active C-7 alkylated tetramate series and tetramate-based prodrugs with incorporation of sugar derivatives required further investigations and optimisation.

Chapter 4 Modification of metal chelation in cysteine-derived tetramate systems - II

This chapter describes the synthesis of cysteine-derived tetramate analogues with functionalisation at C-5 esters (Figure 4.1). Following from Chapter 3, the aim of this functionalisation was to alter the metal binding ability of the tetramate system by sterically modifying groups near the centre of metal chelation. Esters of L-cysteine were synthesised from esterification of L-cysteine followed by reductive cleavage of the disulphide bond. A Weinreb amide was introduced to the side chain of *N*-acylthiazolidines so that the subsequent Dieckmann cyclisation proceeded in the direction to retain the C-5 ester group. The resultant tetramate Weinreb amides allowed diverse functionalisation to access tetramate ketones by Grignard additions and tetramate carboxamides by aminolysis reactions. The incorporation of bulky C-5 esters in tetramate derivatives not only reduced metal binding, as indicated by qualitative comparison of ^1H NMR spectra after column chromatography, but also enhanced their antibacterial potencies against Gram-positive MRSA.

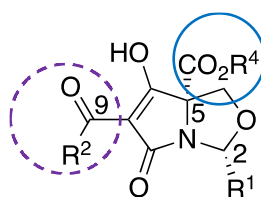


Figure 4.1 Cysteine-derived tetramate analogues with C-5 ester functionalisation.

4.1 Introduction

In Chapter 3, it was established that the C-6, C-8 and C-9 carbonyl groups were directly involved in metal complexation of the tetramate analogues. The synthetic goal of this chapter was to modulate metal chelating ability of tetramate system by alteration of the

functional groups near the centre of metal complexation, i.e. C-5 esters. The hypothesis underlying this chemical modification was that the steric hindrance posed by nearby C-5 esters would discourage metal binding; it was thought that the concave-convex nature of the bicyclic system placed the C-5 in a suitable position to exert such a steric influence. Target compounds were designed with increasing steric bulkiness at C-5, from methyl ester to allyl, isopropyl, cyclohexyl, benzyl, nitro-benzyl and bromo-benzyl esters, the latter selected for its possibility for further functionalisation. Various groups were also introduced at C-9 pendants with side chains such as cyclohexyl, phenyl, phenethyl, styrenyl, and heterocyclic pyridyl and thiazolyl systems in tetramate ketone analogues as well as cyclohexyl and adamantyl amide pendants in tetramate carboxamides (Figure 4.2). The cyclohexyl ketone pendant is a simplified mimic of the *trans*-decalin system found in many tetramate-containing natural products, such as kibdelomycin, altersetin, zopfiellamide A and signermycin B (Figure 1.6, page 14, *vide supra*). Phenethyl and styrenyl derivatives were also structurally similar to naturally occurring tetramates with alkyl ketone side chains such as epicoccarine A, streptolydigin and virgineone (Figure 1.6, page 14, *vide supra*), as well as the endogenous substrates of UPPS such as isopentenyl pyrophosphate (IPP) and farnesyl pyrophosphate (FPP); UPPS is a known biological target of tetramates.⁵¹⁻⁵³ A simple C-9 ethyl ketone series was synthesised to compare structural activity relationships. Heterocyclic rings were introduced at the C-9 ketone pendant to demonstrate versatility of the synthetic route for functionalisation, although it was already established that a hydrophobic core was important at this position for antibacterial activity (Chapter 2, section 2.5, page 64-65, *vide supra*). As detailed in Chapter 2, polar pyridyl rings were also incorporated at C-2 based on the strategy to increase hydrophilicity.

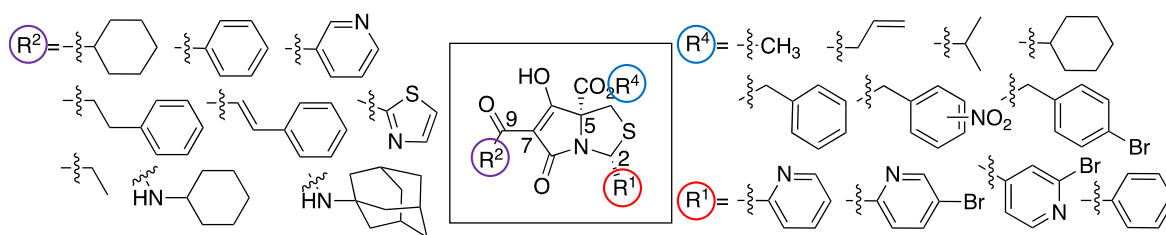
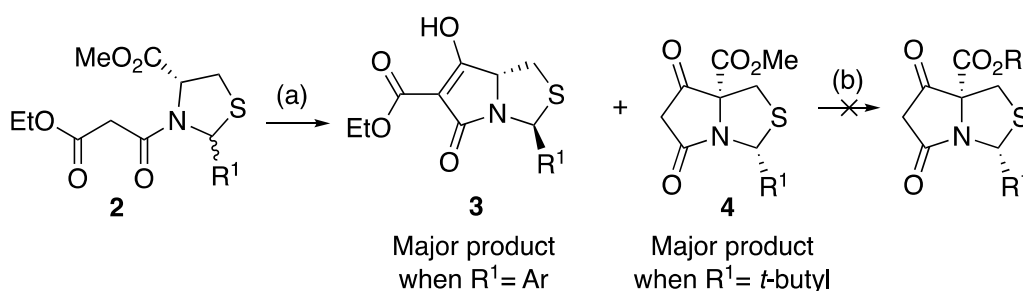


Figure 4.2 Target library of cysteine-derived tetramate analogues.

4.2 Towards modification of C-5 esters in tetramate analogues

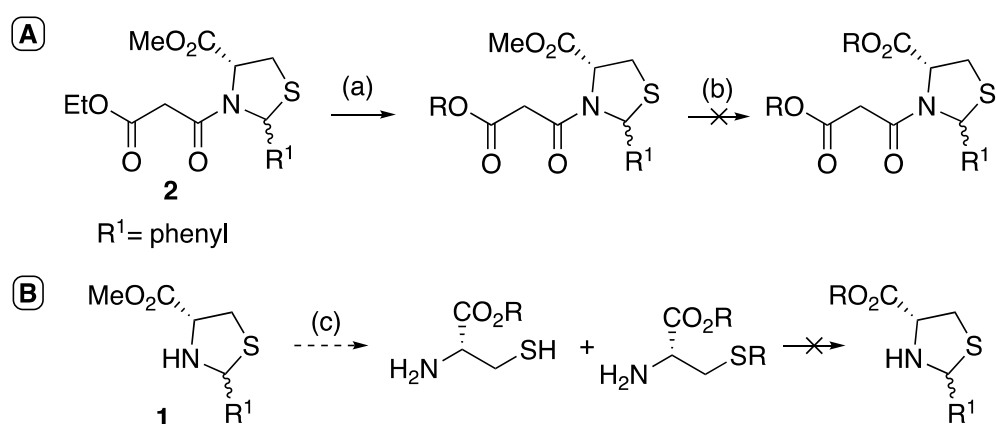
4.2.1 Attempted C-5 functionalisation of the tetramate core and thiazolidines

N-Acylthiazolidines with aromatic C-2 substituents cyclised from the side chain enolate to the C-5 methyl ester to form tetramate esters **3** as the major product, leaving no C-5 methyl esters for further functionalisation (Scheme 4.1). On the other hand, *N*-acylthiazolidines with bulkier C-2 *t*-butyl group cyclised to form the tetramate core **4** with a C-5 methyl ester, which unfortunately could not be directly transesterified, suggesting unusual stability and steric hindrance of the system (Scheme 4.1).



Scheme 4.1 Attempted C-5 ester functionalisation of the tetramate core. *Reagents and conditions:* (a) KO^tBu, THF, reflux, 4 h; (b) ROH, catalytic *p*-TsOH monohydrate or DMAP, toluene, reflux.¹⁶⁷

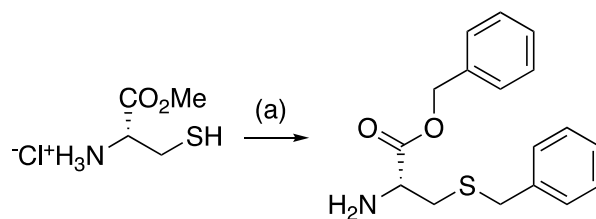
Direct transesterification of the *N*-acylthiazolidine **2** favoured reaction at the less hindered ethyl ester on the side chain (Scheme 4.2 A); transesterification of both ester groups could not be achieved, with the harsh conditions resulting instead in ring opening and degradation of the *N*-acylthiazolidine. Transesterification of the unprotected thiazolidine, known to be less stable than *N*-acylthiazolidine, not surprisingly led to ring opening; the transesterified product of L-cysteine and the product with esterification on the thiol group were detected by mass spectrometry (Scheme 4.2 B).



Scheme 4.2 Attempted C-5 ester functionalisation of thiazolidines. *Reagents and conditions:* (a) ROH, R = *p*-methoxybenzyl, catalytic DMAP, toluene, reflux, o.n.; (b) additional 1 eqv. of ROH, toluene, reflux, o.n.; (c) ROH, R = benzyl or *p*-methoxybenzyl, catalytic *p*-TsOH monohydrate or DMAP, toluene, reflux, o.n.

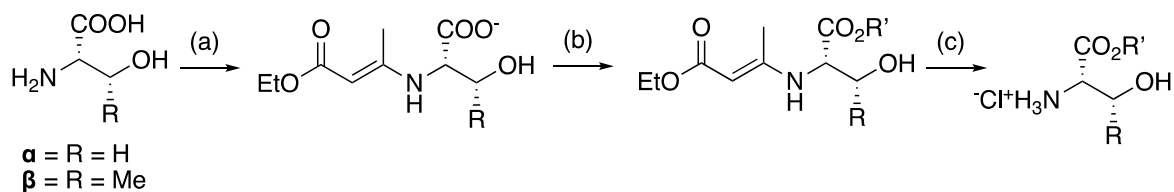
4.2.2 Towards synthesis of L-cysteine esters

However, with the failure to directly functionalise the tetramate core or thiazolidines at the C-5 ester group, focus was shifted to synthesise esters of L-cysteine. With the nucleophilic thiol group present, direct transesterification of L-cysteine methyl ester led to *S*-esterified product (Scheme 4.3).



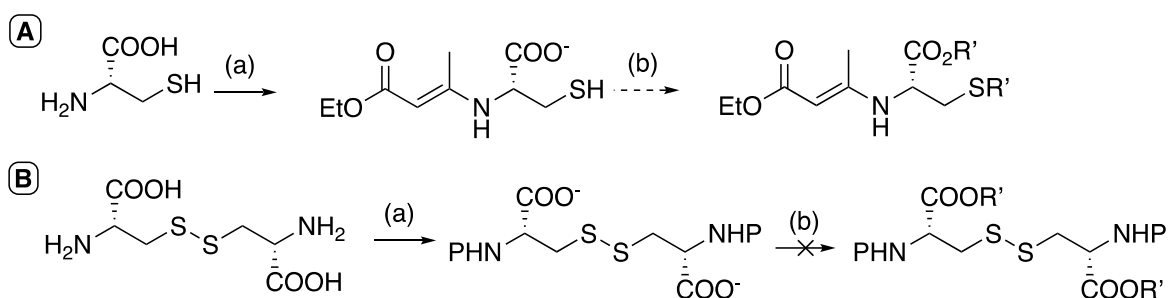
Scheme 4.3 Attempted transesterification of L-cysteine methyl ester. *Reagents and conditions:* (a) benzyl alcohol, catalytic *p*-TsOH monohydrate, toluene, reflux, o.n.

The Moloney group has previously utilised a methodology, modified from procedures published in the literature,¹⁶⁸ to synthesise esters of L-serine and L-threonine, starting from amine protection with ethyl acetoacetate, followed by esterification with alkyl halides under basic conditions and subsequent amine deprotection with methanolic HCl (Scheme 4.4); the enamine function is used as a transient *N*-protecting group.



Scheme 4.4 Synthesis of esters of L-serine and L-threonine. *Reagents and conditions:* (a) *N,N,N,N*-tetramethylguanidine, anhydrous DMF, 0 °C for 30 min followed by addition of ethyl acetoacetate, 0 °C to r.t. 18 h; (b) R'Br, (R' = benzyl, *p*-nitrobenzyl, allyl, butyl), r.t., 24 h; (c) methanolic HCl, 0 °C, 10 min.¹⁶⁸

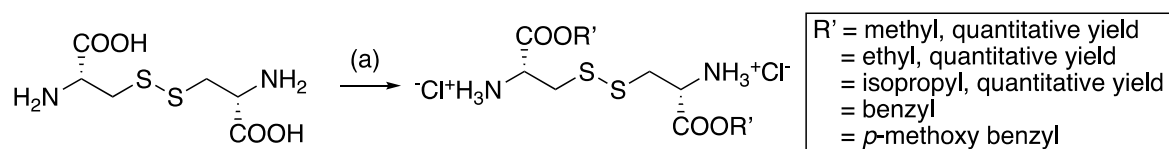
When the same reaction conditions were directly applied to L-cysteine, simultaneous attack from thiol group to the alkyl halide occurred, so that the desired L-cysteine ester product was not detected or isolated (Scheme 4.5 A). When L-cystine was used instead, no successful esterification was seen either (Scheme 4.5 B).



Scheme 4.5 Attempted esterification of L-cysteine and L-cystine. *Reagents and conditions:*

- (a) *N,N,N,N*-tetramethylguanidine, anhydrous DMF, 0 °C for 30 min followed by addition of ethyl acetoacetate, 0 °C to r.t., 18 h, P = protecting group (CH=C(CH₃)CH₂CO₂CH₂CH₃);
- (b) R'Br, (R' = benzyl), r.t., 24 h.

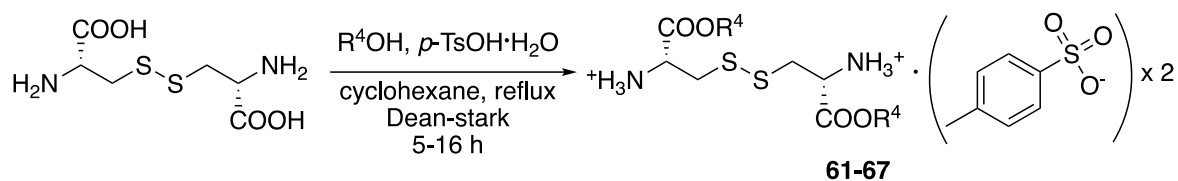
Further attempts were made to synthesise esters of L-cysteine exploiting the attenuated nucleophilicity of sulphur in the disulphide group compared to the unprotected thiol in L-cysteine. Esterification by thionyl chloride and respective alcohols¹⁶⁹ was successful with simple alcohols and occurred in quantitative yield but was more limited with bulkier alcohols such as benzyl alcohol and *p*-methoxybenzyl alcohol, with reaction not being completed and starting material mostly being recovered (Scheme 4.6).



Scheme 4.6 Attempted esterification of L-cystine. *Reagents and conditions:* (a) SOCl₂, R'OH, 0 °C to reflux, 18 h.

Acid-catalysed esterification with *p*-TsOH monohydrate was, however, successful in toluene,^{170,171} with detection of the product by mass spectrometry. The reaction

conditions were optimised to using cyclohexane as a water azeotroping solvent so that it avoided using toluene since racemisation of amino acids has been reported under the esterification conditions in toluene.¹⁷² It also avoided using toxic hazardous solvents such as benzene and CCl₄ that were used in the original procedures.^{173,174} It was reported that benzyl esters of alanine, phenylalanine, tyrosine, phenylglycine, valine, leucine, lysine, serine and methionine were synthesised under the esterification conditions in cyclohexane with high enantiopurity (ee ranging from 96% to 100% in most cases).¹⁷² Under the same conditions, diesters of L-cystine precipitated as *p*-toluenesulphonate salts. The melting points and optical rotation values of L-cystine diesters are recorded in Table 4.1 with reference to available literature reported values.^{175,176,177} Very limited data were available for characterisation of L-cystine diesters, even though all except for **65** and **67** were known previously and used as starting materials for synthesising biologically relevant molecules.¹⁷⁶⁻¹⁸³ As the same reaction conditions were employed for esterification, the high enantiopurity of products previously reported was assumed to be replicated in the synthesis of cystine diesters.

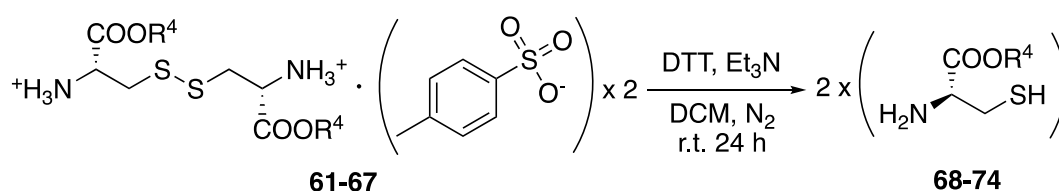
Table 4.1 Synthesis of L-cystine diesters.

R ⁴ =	m.p./°C		[α] _D ²⁵ (c, g/100ml in MeOH)		Yield %
	Measured	Reported	Measured	Reported	
61	136	-	-35.9 (c = 1.0)	-	Quantitative
62	190	155-156 (HCl salt) ¹⁷⁶	-47.8 (c = 0.86)	-	87 to quantitative
63	196	-	-28.9 (c = 1.1)	-	Quantitative
64	178	189 ¹⁷⁷	-25.0 (c = 1.3)	-11.3 (c = 1.0 acetic acid) ¹⁷⁷	Quantitative
65	209	-	-7.4 (c = 1.1)	-	Quantitative
66	199	-	-14.1 (c = 0.95)	-	Quantitative
67	194	-	-12.9 (c = 0.30)	-	Quantitative

Diesters of L-cystine were subsequently reduced at the disulphide bond to produce esters of L-cysteine, which could then be condensed with various aldehydes according to the previously established protocol (Scheme 2.6, page 41, *vide supra*) to access the tetramate system. Initial reduction by sodium borohydride was not successful, with unwanted reduction of the ester groups also being observed, despite it being a relatively mild reducing reagent and being reported in the literature as being suitable for cleaving disulphide bonds in cystine esters.^{179,184} Cleavage of the disulphide bond was achieved with the specific thiol-reducing agent, dithiothreitol (DTT), under weakly alkaline

conditions with Et₃N (Table 4.2).^{185,186} L-Cysteine esters were extracted and purified as free esters and used subsequently to condense with aldehydes. The optical rotation values of L-cysteine esters were tabulated in Table 4.2 with reference to available literature reported values.^{175,187}

Table 4.2 Synthesis of L-cysteine esters by disulphide cleavage of L-cystine diesters.



	R ⁴ =	[α] _D ²⁵ (c, g/100ml)		Yield
		Measured	Reported	
68		-76.0 (c = 1.0, CHCl ₃)	-	82
69		-6.89 (c = 1.0, CHCl ₃)	-	65
70		-34.5 (c = 0.90, CHCl ₃)	-	71
71		-38.0 (c = 0.96, CHCl ₃)	-26.6 ¹⁸⁷ -38.8 ¹⁷⁵ (c = 1, 0.1 N HCl) ^a	83
72		-5.13 (c = 0.54, CHCl ₃)	-	50
73		-15.2 (c = 1.37, CHCl ₃)	-	94
74		-38.0 (c = 1.0, CHCl ₃)	-	53

[a] Optical rotation calculated as the free ester

4.2.3 Synthesis of thiazolidines

Condensation of L-cysteine esters with respective aromatic aldehydes gave the corresponding thiazolidines as a mixture of *cis/trans*-2,5 diastereomers in good yield (Table 4.3). The characteristic and highly conserved H-2 and H-5 chemical shifts were consistent with those previously found in C-5 methyl ester thiazolidines **23a-d** (Table 2.1, page 42, *vide supra*), with H-2 and H-5 chemical shifts both being consistently more downfield in the *trans*-diastereomers. The ratio of *cis* to *trans* diastereomers varied from 0.5 : 1 to 4.5 : 1, which indicated a potential of selectivity towards the desired diastereomer.

Table 4.3 Yield and H-2, H-5 chemical shifts (CDCl₃, 400 MHz) for thiazolidines.

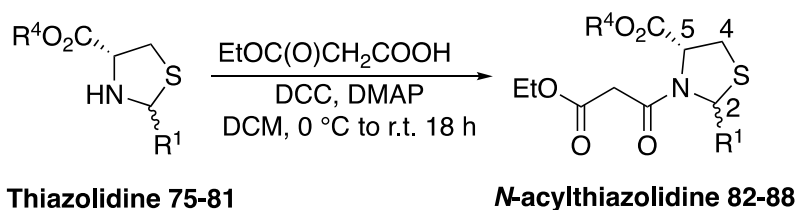
$$\text{H}_2\text{N}-\text{CH}_2-\text{CH}(\text{CO}_2\text{R}^4)-\text{SH} \xrightarrow[\text{Petrol 40/60, reflux with Dean-stark, 18 h}]{\text{R}^1\text{CHO}} \text{Thiazolidine}$$

68-74		Thiazolidines 75-81					
R ⁴ =	R ¹ =	δ_{H} (ppm) for				<i>cis</i> / <i>trans</i>	% Yield
		H-2		H-5			
		<i>cis</i>	<i>trans</i>	<i>cis</i>	<i>trans</i>		
		5.65 (d)	5.86 (d)	4.01 – 4.10 (m)	4.53 – 4.60 (m)	1 : 1	95
		5.57	5.83	3.96 – 4.06 (m)	4.20 – 4.28 (m)	1.8 : 1	68
		5.64 (d)	5.86	3.97 (q)	4.46 (dd)	1 : 1	85
		5.52	5.80	3.89 (dd)	4.05 – 4.12 (m)	1.9 : 1	83
		5.64	5.81	3.99	4.41 (dd)	1.1 : 1	69
		5.52	5.80	3.91 (dd)	4.06 – 4.16 (m)	1.9 : 1	70
		5.61	5.83	4.03 (dd)	4.52 (dd)	1 : 1	45
		5.61	5.82	4.06 (dd)	4.50 (dd)	1 : 1	85
		5.57	5.83	3.99 – 4.08 (m)	4.22 – 4.29 (m)	1.8 : 1	50
		5.66	5.83	4.11 (dd)	4.66 (dd)	1 : 1	74
		5.45	5.71	4.05 – 4.13 (m)		0.5 : 1	77
		5.45	5.72	4.04 – 4.10 (m)		0.5 : 1	72
		5.60	5.80	4.02 – 4.06 (m)	4.51 (dd)	4.5 : 1	55
		5.55	5.80	3.96 – 4.05 (m)	4.25 (dd)	1.7 : 1	91

4.2.4 Synthesis of *N*-acylthiazolidines

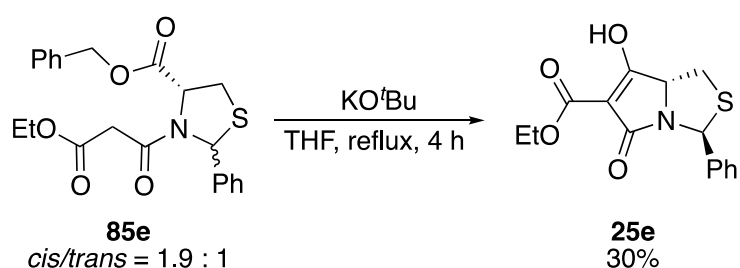
Under the DCC/DMAP coupling conditions, thiazolidines were converted to the corresponding *N*-acylthiazolidines as a mixture of *cis/trans*-2,5 diastereomers in high yield (Table 4.4). Similar to the previously synthesised *N*-acylthiazolidines **24a-d**, each diastereomer appeared as a rotameric pair. The trend of characteristic H-2 chemical shifts was consistent with that observed previously in *N*-acylthiazolidines with C-2 aromatic substituents (Table 2.2, page 43, *vide supra*). In the C-2 phenyl series, the H-2 chemical shifts for the major *trans* rotamers were consistently more downfield; this trend was reversed in minor rotamers. In the C-2 pyridyl series, the H-2 chemical shifts for major *trans* rotamers were consistently more upfield; the trend was preserved in minor rotamers. In the case of **84a**, two diastereomers of the starting thiazolidine **77a** were separated by column chromatography and each was taken for separate *N*-acylation. The reaction with *cis*-**77a** produced a mixture of predominately *cis*-malonamide **84a** with a *cis/trans* ratio of 4.8 : 1, while the reaction with *trans*-**77a** yielded a mixture of predominately *trans*-malonamide with a *cis/trans* ratio of 0.2 : 1. These two separate reactions starting from a single diastereomer produced a mixture of diastereomers, and the changing *cis/trans* ratio from thiazolidines to *N*-acylthiazolidines across the series indicated the existence of an equilibrium between the two isomers under the reaction conditions. It was generally observed that when groups at C-2 and C-5 became bulkier, as in the cases of **85-88**, the steric interaction between them strongly favoured the production of the more stable *cis*-diastereomer, as both groups assumed the pseudoequatorial positions.

Table 4.4 Yield and H-2 chemical shift data (CDCl₃, 400 MHz) for *N*-acylthiazolidines.



	R ⁴ =	R ¹ =	δ_{H} (ppm) for H-2 (s)				<i>cis</i> / <i>trans</i>	% Yield
			<i>cis</i>		<i>trans</i>			
			Major	Minor	Major	Minor		
82a			6.16	6.43	6.13	6.20	3.1 : 1	58
82e			6.15	6.37	6.19	6.33	1.2 : 1	74
83a			6.14	6.40	6.11	6.17	1.4 : 1	71
83e			6.15	6.37	6.17	6.32	1 : 1	97
84a			6.14	6.39	6.10	6.17	1.2 : 1	64
84e			6.15	6.37	6.17	6.32	1.3 : 1	99
85a			6.22	6.48	6.19	6.27	1.7 : 1	49
85c			6.15	6.39	6.13	6.18	1 : 1	68
85e			6.14	6.36	6.18	6.34	1.9 : 1	99
86a			6.20	6.47	6.17	6.24	8.8 : 1	99
86b			6.14	6.20	6.13	6.15	1.5 : 1	71
87b			6.15	6.20	6.14	-	1.1 : 1	88
88c			6.15	6.38	6.13	-	6.5 : 1	80
88e			6.14	6.36	6.18	6.32	2.2 : 1	61

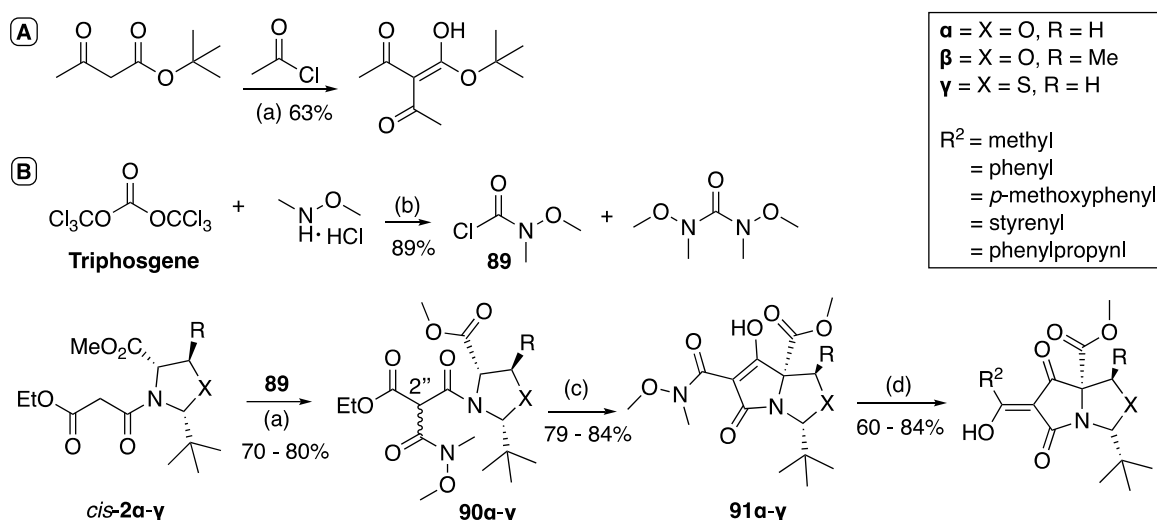
When *N*-acylthiazolidines **85e** with a bulkier C-5 ester was taken directly for Dieckmann cyclisation, it unfortunately cyclised from the more stable side chain enolate to the C-5 ester, giving tetramate ester **25e** as the major product with loss of the C-5 ester (Scheme 4.7). The fact that *N*-acylthiazolidine **43e** which was *C*-methylated at the side chain cyclised in a different direction to retain the C-5 ester (Scheme 3.12, page 93, *vide supra*) suggested functionalisation at the side chain could bring changes in relative steric hindrance and stability of the enolates, switching the direction of Dieckmann cyclisation to favour the formation of desired target compounds with C-5 esters retained.



Scheme 4.7 Attempted Dieckmann cyclisation of *N*-acylthiazolidine **85e**. (The *trans* diastereomer of **85e** cyclised to form **25e** while the *cis* diastereomer was recovered.)

4.2.5 Towards synthesis of tetramate Weinreb amides with different C-5 esters

Instead of simple alkyl groups at the *N*-acyl side chain with no further opportunity of functionalisation, a Weinreb amide was introduced under Sucunza's acylation conditions, which were initially used to acetylate a dicarbonyl with acetyl chloride (Scheme 4.8 A).¹⁸⁸ The same acylation conditions were used previously in the Moloney group to synthesise *C*-acylated *N*-acyloxazolidines or thiazolidines **90 α - γ** with a *C*-2 *t*-butyl group (Scheme 4.8 B).¹¹² Compounds **90 α - γ** were cyclised to tetramate Weinreb amides **91 α - γ** , which were further functionalised under the Grignard conditions to produce tetramate ketone analogues (Scheme 4.8 B).¹¹²



Scheme 4.8 Previous work of introduction of Weinreb amide in the Moloney group following Sucunza's acylation conditions.¹¹² *Reagents and conditions:* (a) pyridine, MgBr₂, DCM, 0 °C to r.t., 18 - 24 h; (b) pyridine, DCM, -78 °C to r.t., 16 h;¹⁸⁹ (c) DBU, THF, r.t., 24 h; (d) R²MgBr, dry THF, -15 °C, 1 h.

Successful C-acylation on the side chain of the *N*-acylthiazolidines **82-88** was reproduced under the same conditions (Table 4.5). Consistent with the previous observations, the side chain tricarbonyl system existed in the keto-form, indicated by the presence of H-2'' singlet in ¹H NMR spectra. After 24 hours, reactions were not completed, with starting malonamides enriched in *trans*-diastereomers being recovered, even with 1.5 equivalents of the acid chloride **89**. For products **92-99**, each was an approximately equal ratio mixture of C2'' *R/S* diastereomers with characteristic H2'' chemical shifts (Table 4.5). The *cis/trans* diastereomeric ratio in the product ranged from 2.1 : 1 to exclusive production of *cis* diastereomer in most cases. The relative stereochemistry was supported by NOE analysis (Figure 4.3), while 2D NOESY also confirmed the presence of rotamers in the mixture. The introduction of an additional chiral centre at C-2'' to a mixture of *cis/trans* *N*-acylthiazolidines and rotameric behaviour of malonamides added to the complexity of

characterisation. It was previously reported that the *cis*-product **90 α - γ** was a mixture of diastereomers at C-2'' with ratios ranging from 1.4 : 1 to 2 : 1.¹¹² The diastereomers potentially present in the product mixture were not separable by flash column chromatography and the mixture was used directly for Dieckmann cyclisation.

In the case of **95a**, the predominately *cis* mixture of **84a** (*cis/trans* = 4.8 : 1, page 122, *vide supra*) produced *cis*-**95a** exclusively under the acylation conditions in 68% yield. The reaction with the predominately *trans* mixture of **84a** (*cis/trans* = 0.2 : 1, page 122, *vide supra*) was less straightforward. Unreacted *trans* starting material was recovered in 42% yield; product was formed in 38% yield as a mixture of *cis/trans* (0.4 : 1) and C2'' *R/S* (0.8 : 1) diastereomers. This result suggested that the *cis*-malonamides reacted favourably under the acylation conditions and there could be interconversion between *cis/trans* diastereomers to favour the *cis*-2,5 products.

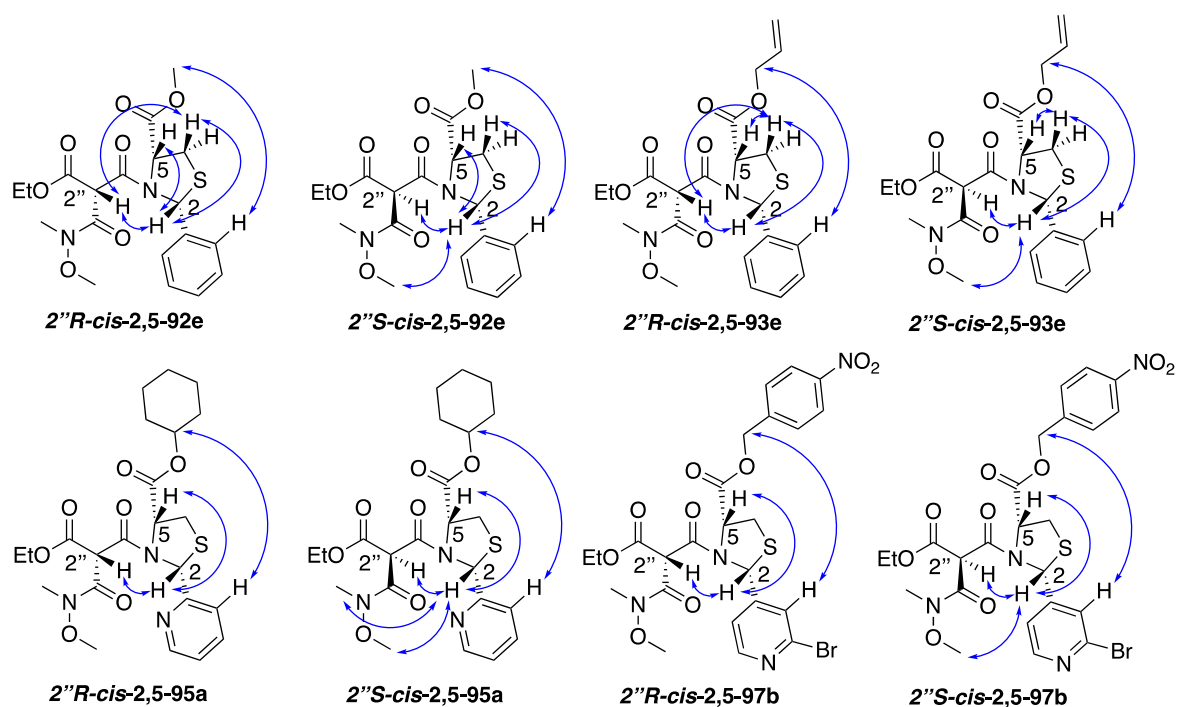
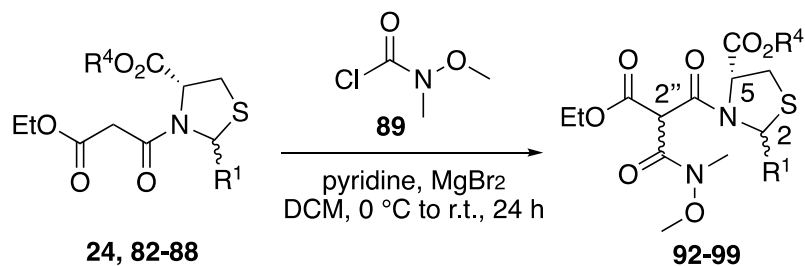


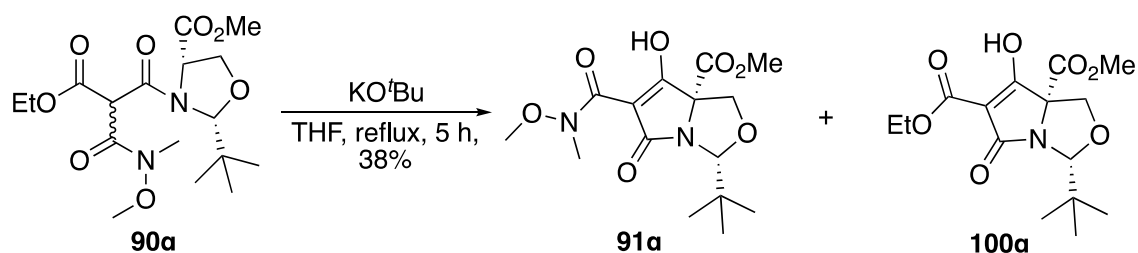
Figure 4.3 NOE analysis to confirm relative *cis*-2,5 stereochemistry and assign *R/S* at C2''.

Table 4.5 Yield and H-2, H-5 chemical shifts (CDCl₃, 400 MHz) for C-acylated thiazolidines.



	R ⁴ =	R ¹ =	δ_{H} (ppm)				Cis/ trans	R/S	% Yield
			H2 (major)		H2''				
			cis	trans	C2''R	C2''S			
92e	CH ₃		5.77 5.95	-	4.44 4.35	cis	1 : 1	77	
93a			5.95 6.00	-	4.62 4.48	cis	0.8 : 1	47	
93e			5.82 6.00	-	4.49 4.39	cis	1 : 1	54	
94a			5.96 6.02	-	4.62 4.49	cis	0.7 : 1	54	
94e			5.81 6.00	-	4.49 4.41	cis	0.8 : 1	61	
95a			5.94 6.00	(6.27 6.30)	4.60 4.48	(-)	0.7 : 1	68	
95e			5.82 6.01	-	4.49 4.40	cis	0.8 : 1	51	
96a			5.98 6.02	-	4.64 4.51	cis	0.7 : 1	53	
96c			5.95 5.98	6.21 6.24	4.64 4.52	2.1 : 1	0.7 : 1	40	
96e			5.81 5.99	-	4.46 4.38	cis	0.8 : 1	70	
97a			6.02 6.04	-	4.69 4.59	cis	0.7 : 1	57	
97b			5.89 6.09	-	4.49 4.48	cis	1 : 1	65	
98b			5.86 6.11	-	4.51 4.48	cis	1 : 1	40	
99c			5.95 5.97	-	4.64 4.53	cis	0.7 : 1	69	
99e			5.82 5.99	-	4.46 4.37	cis	1 : 1	49	

It was noted previously that Dieckmann cyclisation of **90a** with KO^tBu in THF under reflux led to an inseparable 1 : 1 mixture of **91a** and **100a**, both cyclised from C-5 enolate to the side chain ethyl ester (Scheme 4.9), while cyclisation with DBU in THF at room temperature exclusively produced the desired Weinreb amide **91a-γ** in high yield (Scheme 4.8 B, page 125, *vide supra*).¹¹²



Scheme 4.9 Previously reported cyclisation of **90a** under the basic conditions of KO^tBu.¹¹²

Under the modified reaction conditions with DBU, successful Dieckmann cyclisation was achieved generally in good yield, leading to the desired tetramate Weinreb amides **101-109** with different C-5 esters (Table 4.6). The presence of basic C-2 pyridyl groups and hence the overall increase in product polarity added to the difficulty of acidic work-up after the reactions, lowering yields significantly. For cyclisation with **93a** and **94a**, which contained C-2 unsubstituted pyridyl ring, although product mass ions were detected by mass spectrometry, no product could be extracted. Increasing lipophilicity of groups at C-2 and C-5 improved the ease of handling.

Under Dieckmann cyclisation conditions, the *cis* diastereomer of tricarbonyl malonamides cyclised from the C-5 enolate onto the side chain carbonyl by placing the C-2 substituents at the less hindered *exo*-face of the bicyclic ring, producing tetramate derivatives with C-5

ester groups retained. In the reaction with *trans*-**95a**, no tetramate product was observed and starting material was recovered. It was interesting to note the efficiency and chemoselectivity of the synthetic route, since with bulky C-5 esters, the pathway demonstrated strong preference towards the *cis*-diastereomer, which was the only diastereomer that cyclised under the Dieckmann cyclisation with C-5 ester retained. The basic conditions with DBU also favoured exclusively the elimination of ethoxide rather than the amine from the side chain, retaining the Weinreb amide for diverse late-stage functionalisation at C-7.

Weinreb amides **101-109** were completely enolic, with no C-7 proton observed on ^1H NMR spectra. The Weinreb amides **101-109** also appeared as a single diastereomer at C-2/C-5 with characteristic and consistent H-2 chemical shifts (Table 4.6). The *cis* relationship between C-5 ester and C-2 aromatic rings were supported by NOE analysis (Figure 4.4). The *endo*-enolic tautomeric form **A** was assigned to Weinreb amides **101-109**, supported by HMBC analysis (Figure 4.5) and by comparison of carbonyl peaks with previously synthesised **91 α - γ** .¹¹²

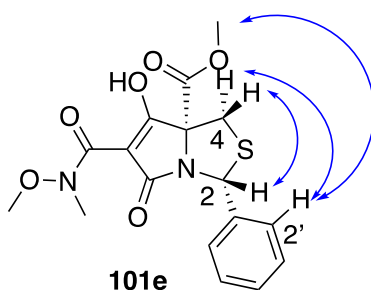


Figure 4.4 NOE analysis of **101e**.

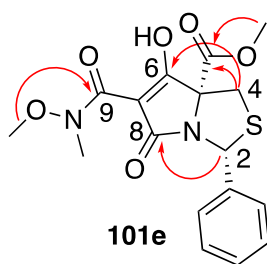
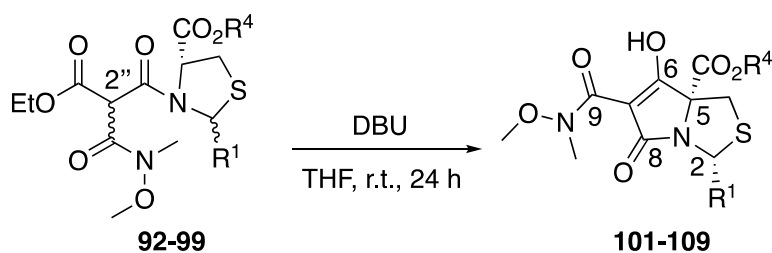


Figure 4.5 HMBC correlation analysis of **101e**.

In the reactions with **97b** and **98b**, which only contained C-5 nitrobenzyl esters, the product was unexpectedly a 1 : 1 inseparable mixture of tetramate Weinreb amides with respective C-5 nitrobenzyl ester and ethyl ester (by-product **107b** formation, Table 4.6). The by-products could arise from transesterification with the eliminated ethoxide under the basic conditions of DBU. The increasing bulkiness of C-5 esters could put strain on the hindered bicyclic tetramate system, giving rise to increased structural instability. With the nitrobenzyl system, the reactions favoured products with a much smaller C-5 ethyl ester. Similar by-product formation was observed from the crude of **105c** and **109c/e** but to a much less extent and desired products could be purified with column chromatography. The by-product formation with bulky C-5 esters limited the scope of esters prior to Dieckmann cyclisation, which made **109c/e** more valuable as their bromo-substituted ester allowed expansion of ester diversity via late-stage functionalisation.

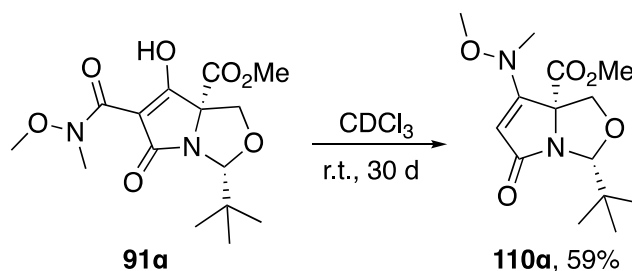
Table 4.6 Yield, H-2, C-6, C-8, C-9 chemical shifts (CDCl₃) for tetramate Weinreb amides.



	R ⁴ =	R ¹ =	δ (ppm)				% Yield
			H-2	C-6	C-8	C-9	
101e			6.32	185.5	176.9	166.3	93
102e			6.32	185.6	177.0	166.3	86
103e			6.32	185.9	-	166.4	85
104a			6.38	-	-	166.5	32
104e			6.32	185.8	176.6	166.2	63
105a			6.39	186.4	176.7	166.4	28
105c			6.29	186.2	-	166.2	40
105e			6.30	-	-	166.3	70
106a			6.40	186.0	177.0	166.3	20
106b			6.18	-	-	166.1	41 (1 : 1)
107b			6.18	-	-	166.0	
108b			6.19	-	-	166.1	45 (1 : 1 with 107b)
109c			6.31	-	177.2	166.3	35
109e			6.31	185.3	177.2	166.3	56

In tetramate Weinreb amides, C-6 and C-8 signals were weak in the ¹³C NMR spectrum, which could be a result of a combination of tautomeric and rotameric behaviours.¹¹²

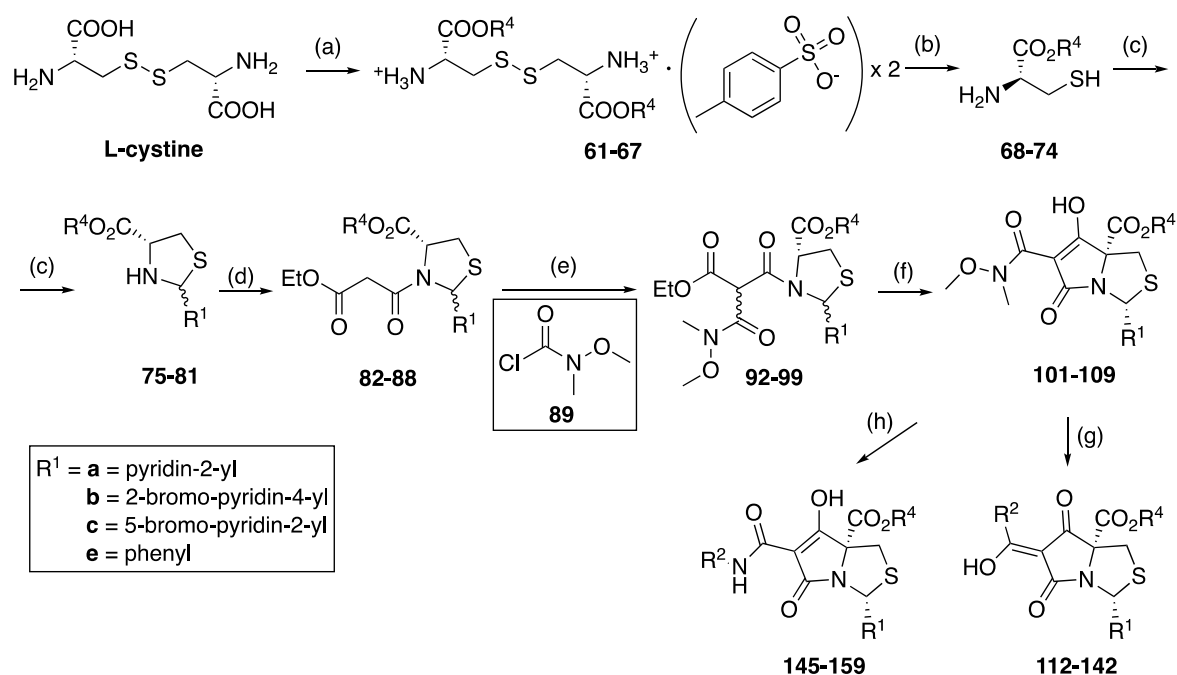
In addition, it was noted that Weinreb amides were not stable when kept in solution, although they were relatively stable to column chromatography and when kept solventless in the fridge. A second set of minor peaks could appear when Weinreb amides were kept in CDCl_3 overnight for NMR characterisation. The instability of Weinreb amides was also observed previously, and for example complete degradation of Weinreb amide **91 α** in 30 days left enamine **110** as the only isolated product in 59% yield (Scheme 4.10); this potentially resulted from hydrolysis, decarboxylation and subsequent reaction of the β -ketolactam with the released amine.¹¹²



Scheme 4.10 Previously observed degradation of tetramate Weinreb amide **91 α** .¹¹²

4.3 Functionalisation of tetramate Weinreb amides

The presence of the C-7 Weinreb amide allowed further functionalisation by introducing C-7 acyl substituents using Grignard reactions and C-7 amides using aminolysis. The synthetic route from L-cystine to access target tetramate analogues with C-5 ester modifications is summarised in Scheme 4.11.

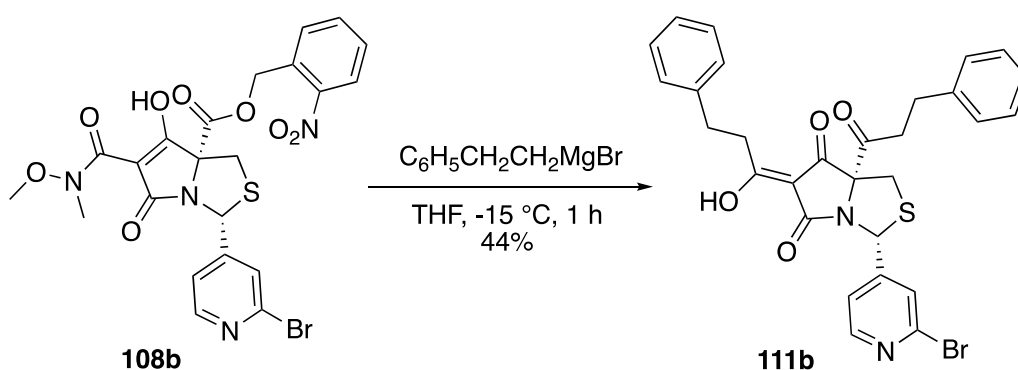


Scheme 4.11 Synthetic route to target tetramate analogues with C-5 ester functionalisation. *Reagents and conditions:* (a) *p*-TsOH monohydrate, $R^4\text{OH}$, cyclohexane, reflux with Dean-stark, 5 -16 h; (b) DTT, Et_3N , DCM, N_2 , r.t., 24 h; (c) $R^1\text{CHO}$, Et_3N , petrol 40/60, reflux with Dean-stark, 18 h; (d) mono-ethyl malonate, DCC, DMAP, DCM, 0 °C to r.t., 18 h; (e) *N*-methoxy-*N*-methylcarbamoyl chloride **89**, dry pyridine, MgBr_2 , DCM, 0 °C to r.t., 18 - 24 h; (f) DBU, dry THF, r.t., 24 h.; (g) $R^2\text{MgBr}$ (prepared fresh with $R^2\text{Br}$, Mg, dibromoethane, dry THF, reflux, 1 h), dry THF, -15 °C, 1 h or o.n.; (h) $R^2\text{NH}_2$, THF/toluene, reflux, 18 h.

4.3.1 Synthesis of tetramate ketone analogues by Grignard reactions

Aryl, alkyl and alkenyl Grignard reagents were prepared by direct metalation of the corresponding bromide with magnesium turnings and dibromoethane as an 'entrainment' agent. Successful C-7 acylation of Weinreb amides was achieved under the Grignard reaction conditions with high chemoselectivity (Table 4.7), with no reactions at the C-5 esters observed, except in the Grignard addition to **108b** which gave some C-5 ketone

formation (Scheme 4.12). This unexpected reaction was probably due to the relatively unstable C-5 2-nitrobenzyl ester in a constrained space. The high chemoselectivity of Grignard addition was attributed to both the hindered nature of the bicyclic tetramate system and the stability of a magnesium-chelated intermediate with the Weinreb amide, avoiding over-addition to tertiary alcohols.¹⁹⁰ It was also reported that this Mg-chelated intermediate was only stable at low temperature and thus required a low-temperature reaction quench.¹⁹⁰



Scheme 4.12 Product formation with C-5 and C-7 ketones from Grignard addition to **108b**.

Reagents and conditions: $\text{PhCH}_2\text{CH}_2\text{MgBr}$, dry THF, $-15\text{ }^\circ\text{C}$, 1 h.

In the synthesised tetramate ketones, the *cis* relative stereochemistry at C-2/C-5 was supported by NOE analysis (Figure 4.6). With consistent H-2 chemical shifts across the series, *cis*-2,5 stereochemistry was assumed to be conserved from the starting tetramate Weinreb amides under the Grignard conditions.

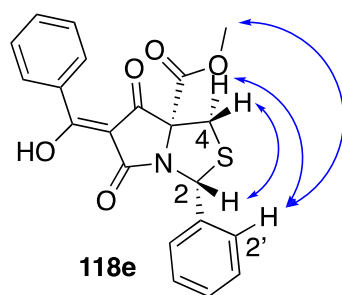


Figure 4.6 An example of NOE analysis for tetramate ketone analogues.

Table 4.7 Yield, H-2, C-6, C-8 and C-9 chemical shifts (CDCl₃) for tetramate ketones.

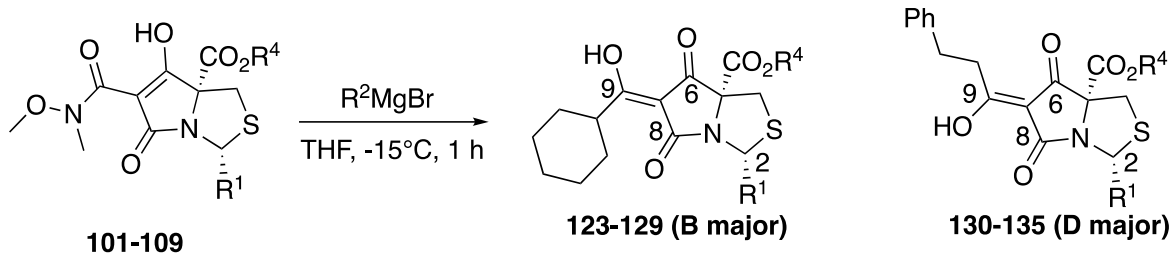
101-109

112-117 (B major)

118-122 (D major)

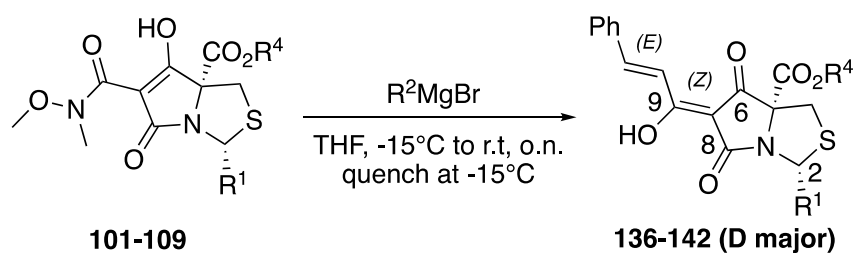
	R ² =	R ⁴ =	R ¹ =	δ (ppm)				AB /CD	% Yield
				H-2	C-6	C-8	C-9		
112e		-CH ₃		6.42	192.3	175.6	187.3	4.1	71
				6.47	-	-	-	: 1	
113e				6.42	192.3	175.8	187.3	3.7	45
				6.46	-	-	-	: 1	
114e				6.41	192.0	175.9	187.6	4.2	40
				6.46	-	-	-	: 1	
115e				6.40	191.9	175.8	187.7	4.5	80
				6.44	-	-	-	: 1	
116e				6.40	192.3	175.9	187.3	4.2	43
				6.44	-	-	-	: 1	
117e				6.40	192.5	175.9	187.3	3.3	42
				6.42	-	-	-	: 1	
118e		-CH ₃		6.49	185.9	177.7	182.9	CD	33
119e				6.49	189.9	177.1	181.5	0.2	46
				6.40	185.9	177.9	182.9	: 1	
120e				6.49	186.2	177.9	182.6	CD	62
121e				6.48	186.4	177.9	182.5	CD	53
122e				6.47	185.9	178.0	182.9	CD	30

Table 4.7 Continued Yield, H-2, C-6, C-8, C-9 chemical shifts (CDCl₃) for tetramate ketones.



	R ² =	R ⁴ =	R ¹ =	δ (ppm)				AB /CD	% Yield
				H-2	C-6	C-8	C-9		
123e		CH ₃		6.41	195.3	176.1	187.1	3.8	35
				6.44	-	-	182.4		
124b				6.28	195.9	176.4	186.3	3.3	33
				6.30	-	-	-		
125e				6.41	195.3	176.3	187.1	4.0	39
				6.44	-	-	-		
126e				6.40	195.1	176.4	187.4	4.0	30
				6.43	-	-	-		
127e				6.39	195.0	176.4	187.6	4.3	57
				6.42	-	-	-		
128c				6.35	195.7	176.8	187.0	3.7	29
				6.38	194.3	172.9	186.0		
128e				6.38	195.3	176.4	187.1	4.6	37
				6.42	-	-	-		
129e				6.38	195.5	176.4	186.9	5.1	36
				6.41	-	-	-		
130e		CH ₃		6.40	189.8	175.3	187.1	CD	40
131e				6.41	191.5	174.7	188.1	0.8	33
				6.40	189.8	175.5	187.2		
132e				6.41	-	-	-	0.1	45
				6.40	189.5	175.6	187.5		
133e	Phenethyl			6.42	-	-	-	0.2	73
				6.40	189.4	175.6	187.6		
134e				6.38	189.8	175.6	187.1	CD	40
135e				6.43	-	-	-	0.2	31
				6.38	190.1	175.6	187.1		

Table 4.7 Continued Yield, H-2, C-6, C-8, C-9 chemical shifts (CDCl₃) for tetramate ketones.



	R ² =	R ⁴ =	R ¹ =	δ (ppm)				AB /CD	% Yield
				H-2	C-6	C-8	C-9		
136e		-CH ₃		6.51	-	-	-	0.26 : 1	58
				6.46	187.5	176.0	176.2		
137b				6.36	-	-	-	0.34 : 1	31
				6.32	186.7	176.3	176.6		
138e				6.51	196.3	175.0	176.7	0.17 : 1	58
				6.46	187.5	176.1	176.2		
139e	Styrenyl ^a			6.50	196.7	-	176.5	0.28 : 1	77
				6.46	187.7	176.0	176.3		
140e				6.49	196.9	-	176.5	0.28 : 1	53
				6.45	187.9	175.9	176.3		
141c				6.44	196.1	175.5	177.0	0.43 : 1	29
				6.40	187.4	176.5	176.7		
141e				6.49	-	-	-	0.26 : 1	55
				6.44	187.4	176.2	176.3		
142e				6.49	-	-	-	0.23 : 1	59
				6.44	187.3	176.2	176.3		

[a] The *E* stereochemistry of the styrenyl ketone pendant was confirmed by the *J* coupling value of H-1'' and H-2'' (consistently at *J* = 16.0 Hz, indicating the *trans* relationship between the two protons) on the side chain.

Similar to tetramate carboxamides **27-30** (Figure 2.14, page 59, *vide supra*), tautomers could exist for tetramate ketone analogues **112-142** (Figure 4.7). The presence of two H-2 chemical shifts for each tetramate ketone analogue and the distinctive two sets of carbonyl chemical shifts across the series also suggested the existence of tautomers (Table 4.7, page 135-137, *vide supra*).

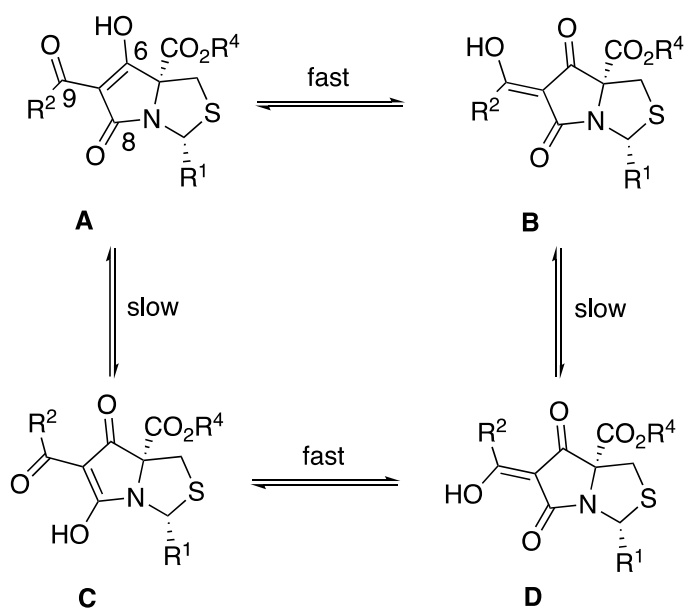


Figure 4.7 Tautomeric behaviour of tetramate ketones. Adapted with permission from the Royal Society of Chemistry (Panduwawala, T. D.; Iqbal, S.; Thompson, A. L.; Genov, M.; Pretsch, A.; Pretsch, D.; Liu, S.; Ebright, R. H.; Howells, A.; Maxwell, A.; Moloney, M. G. *Org. Biomol. Chem.* **2019**, *17*, 5615–5632.) Copyright 2019.

The assignment of tautomeric forms was assisted by HMBC analysis (Figure 4.8) for allocating C-6, C-8 and C-9 carbonyl shifts as well as deduction from differing inductive effects and hydrogen bonding, which indicated the *Z*-*exo*-enolic **D** as the major or exclusive tautomeric form in the $R^2 =$ phenyl, phenethyl and styrenyl series while *E*-*exo*-enolic form **B** as the major tautomer in the $R^2 =$ ethyl and cyclohexyl series. The C-6 chemical shifts would be the most downfield in *exo*-enolic form **B**, due to existence of hydrogen bonding with C-9 enol, followed by **C/D** forms and lowest in *endo*-enolic form **A**, due to resonance, although **A/B** are internal tautomers and NMR signals are averaged out. For the major forms, tetramate ketones with $R^2 =$ ethyl and cyclohexyl consistently exhibited more downfield C-6 chemical shifts than the $R^2 =$ phenyl, phenethyl and styrenyl series (Table 4.7, page 135-137, *vide supra*), which supported the assignment of the

former as tautomeric form **B** and the later as form **D**. The C-6 chemical shifts in tetramate Weinreb amides **101-110** assigned as *endo*-enolic form **A** (Table 4.6, page 131, *vide supra*) were indeed the lowest as compared to the two series of C-6 chemical shifts among tetramate ketones. C-8 and C-9 chemical shifts of tautomeric pair **B/D** were very similar, with C-8 being generally more downfield in **D** because of hydrogen bonding. C-9 chemical shifts in the R² = styrenyl series were more upfield than others due to the extended conjugation system of the styrenyl side chain. The trend observed in carbonyl chemical shifts and the assignment of tautomeric forms was consistent to those in previously synthesised tetramate ketones with C-2 *t*-butyl group (Scheme 4.8 B, page 125, *vide supra*). Besides solvents used for NMR analysis,^{106,112} it was evident that the ratio of tautomeric forms also highly depended on the nature of C-9 ketone pendant.

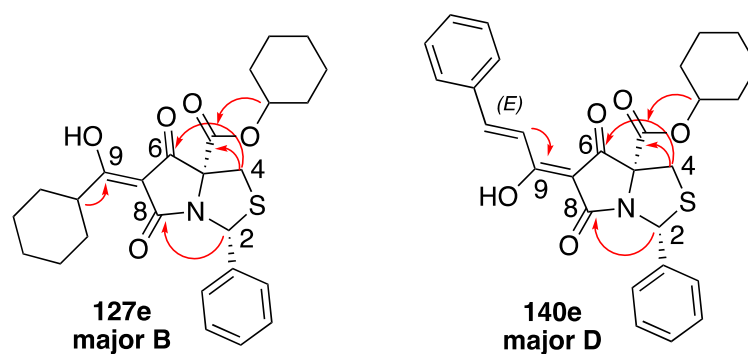
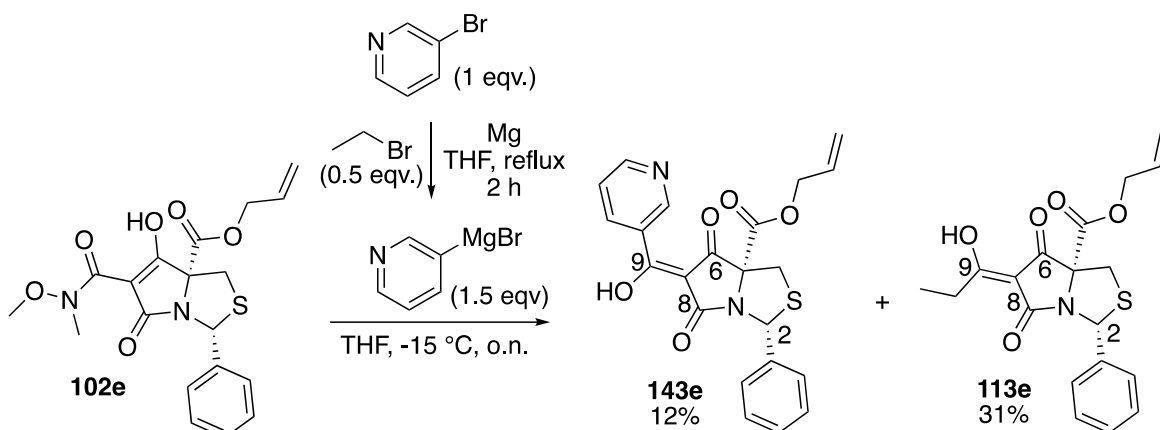


Figure 4.8 HMBC correlation analysis of **127e** and **140e**.

In order to expand the diversity of functionalisation at C-7, heterocyclic Grignard reagents such as pyridyl and thiazolyl systems were prepared for Grignard addition. Pyridyl magnesium bromide was prepared with ethyl bromide as an ‘entrainment’ agent and successful Grignard addition was achieved subsequently (Scheme 4.13). It was reported in the literature that the yield of Grignard reaction increased by increasing the ratio of ethyl

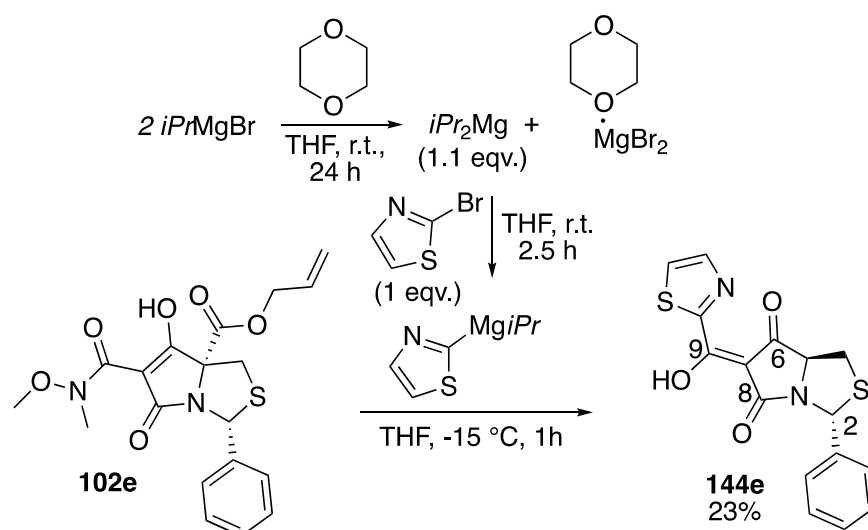
bromide to 3-bromopyridine from 1 : 1 to 3 : 1, with an excess of magnesium and electrophile.¹⁹¹ This method was however not optimal with tetramate Weinreb amides as it generated the corresponding C-9 ethyl tetramate ketone as by-product in every reaction and wasted the “valuable” tetramate intermediate.



Scheme 4.13 Incorporation of 3-pyridyl ring at ketone pendant of tetramate analogues.

Another widely used method of preparing organomagnesium reagents was through halogen-metal exchange.¹⁹² However, it was noted that this method developed by Knochel et al by exchange with *iPrMgBr* was efficient only if the heteroaryl ring bears an additional electron-withdrawing group.^{192,193} It was reported that perfluorinated 4-bromopyridine underwent a bromine-magnesium exchange with *iPrMgBr* at -40 °C within 0.5 h but the unsubstituted 2-bromothiazole underwent the exchange reaction only at room temperature and requires the use of *iPr₂Mg*.¹⁹³ The diisopropylmagnesium salt was prepared by disproportionation of *iPrMgBr* via precipitation with anhydrous 1,4-dioxane and the supernatant was used directly for exchange.^{194,195} Subsequent Grignard addition to Weinreb amide was observed but the resultant Grignard reagent *iPrMg*-thiazole appeared to have different reactivity than other organomagnesium bromides as the C-5

ester was lost in the product under the same Grignard conditions (Scheme 4.14). *Exo*-enolic tautomeric form **D** was assigned to both tetramate ketones **143e** and **144e** based on comparison of the carbonyl shifts with previously synthesised ketone series (Table 4.8). The preparation of heterocyclic Grignard reagents to functionalise tetramate Weinreb amides would require optimisation but no further attempt was made, since as established by Chapter 2 and by previous work in the Moloney group,¹¹⁰ a hydrophobic core was probably necessary at C-9 pendant for tetramate analogues to be antibacterial active.



Scheme 4.14 Incorporation of thiazole ring at C-7 of tetramate analogues.

Table 4.8 Yield and H-2, C-6, C-8, C-9 chemical shifts (CDCl₃) for **143e** and **144e**.

	R ² =	R ⁴ =	R ¹ =	δ (ppm)				AB /CD	% Yield
				H-2	C-6	C-8	C-9		
143e				6.47	189.6	176.2	181.6	CD	12
144e		-		6.48	189.2	173.0	177.9	CD	23

4.3.2 Synthesis of tetramate carboxamides analogues by aminolysis reactions

Besides C-7 ketones, C-7 carboxamides can also be accessed from tetramate Weinreb amides by refluxing them with the corresponding amines. A library of tetramate carboxamides was synthesised via this synthetic route with additional C-5 esters and opposite stereochemistry at C-2 and C-5 to the tetramate carboxamides **27-30** (page 57, *vide supra*). Interestingly, tetramate carboxamides **145-159** exhibited two distinctive sets of H-2 shifts and carbonyl peaks for each compound, which were of approximately equal ratio (Table 4.9). The two sets of H-2 peaks were present in crude ^1H NMR spectra, indicating that they did not arise from column chromatography or post-column acid wash. 2D NOESY ruled out rotameric behaviours as the two sets of peaks did not show rapid dynamic exchange while 1D NOE analysis supported the same *cis*-relative stereochemistry at C-2 and C-5 for both (Figure 4.9). The assignment of carbonyl peaks was assisted by HMBC correlations, which showed that each of the two H-2 shifts correlated with a different C-8 carbonyl (Figure 4.10). The only plausible explanation for the two sets of chemical shifts from a single reaction was tautomeric behaviour, which was previously observed with tetramate carboxamides **27-30** but with different ratios in CDCl_3 (page 61, *vide supra*). The range of carbonyl chemical shifts was comparable to that of **27-30** and the consistent downfield trend in carbonyl peaks in tautomeric pair **CD** than **AB** was also similar to that observed with carboxamides **27-30**. This tautomeric behaviour is also solvent dependent. In methanol, only one set of chemical shifts was observed in both ^1H and ^{13}C NMR spectra (Figure 4.10, C and D), which appeared to be that of an averaged hybrid structure due to equilibrium of the tautomeric forms. Similar equilibration was observed in other polar solvents such as CD_3CN and $\text{THF-}d_8$ but with broader signals, suggesting different rates of the solvent-assisted proton-transfer reactions.

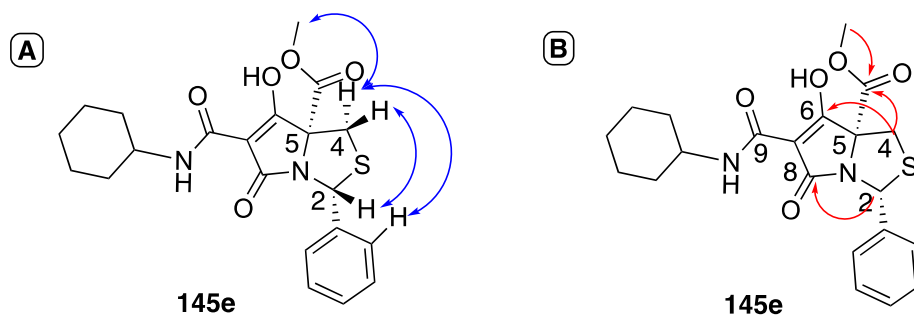
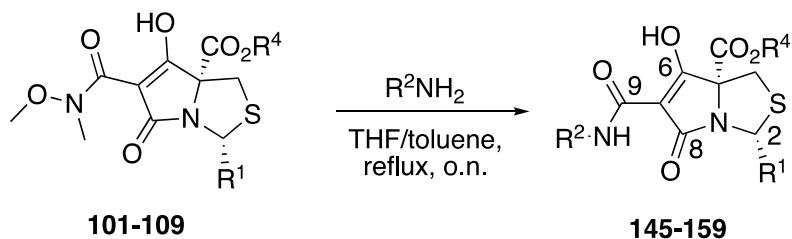



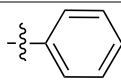

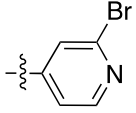
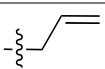
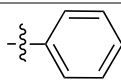

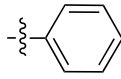
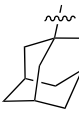
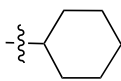
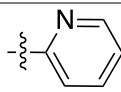
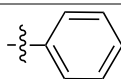
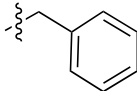
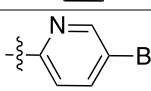
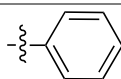
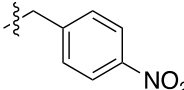
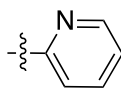
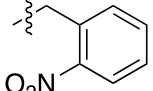
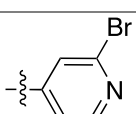
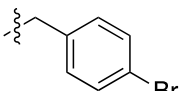
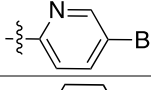
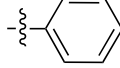
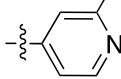
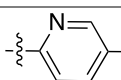
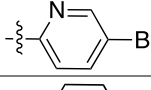
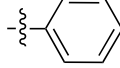
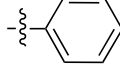
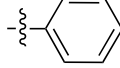
Figure 4.9 (A) An example of NOE analysis suggesting *cis*-2,5 stereochemistry of **145e**; (B) an example of HMBC correlation analysis for assignment of carbonyl peaks.

Table 4.9 Yield, H-2, C-6, C-8, C-9 chemical shifts (CDCl₃) for tetramate carboxamides.



	R ² =	R ⁴ =	R ¹ =	δ (ppm)				AB /CD	% Yield
				H-2	C-6	C-8	C-9		
145e		-CH ₃		6.27	187.1	173.2	165.8	1 : 1	30
				6.31	189.0	178.4	165.9	1	
146e				6.26	187.0	173.3	165.7	0.9	48
				6.31	188.8	178.5	165.9	: 1	
147e				6.26	187.4	173.4	165.7	1.1	49
				6.31	188.6	178.7	165.9	: 1	
148e				6.25	187.5	173.4	165.7	1.1	61
				6.30	188.6	178.7	165.9	: 1	
149c				6.24	187.1	173.5	165.8	0.9	58
				6.27	189.4	178.6	165.8	: 1	
149e				6.25	187.1	173.5	165.8	0.9	35
				6.29	188.9	178.7	165.9	: 1	
150e				6.25	186.9	173.4	165.8	0.9	47
				6.29	189.0	178.6	165.9	: 1	

Table 4.9 Continued Yield, H-2, C-6, C-8, C-9 chemical shifts (CDCl₃) for tetramate ketones.

	R ² =	R ⁴ =	R ¹ =	δ (ppm)				AB /CD	% Yield
				H-2	C-6	C-8	C-9		
151e				6.26	187.0	173.4	166.7	0.9 : 1	56
				6.31	189.4	178.6	166.8		
152b				6.14	186.6	173.5	166.7	1 : 1	52
				6.18	189.7	178.5	166.8		
153e				6.26	187.0	172.6	166.7	1 : 1	46
				6.31	189.3	178.8	166.8		
154e				6.24	187.2	173.5	166.5	1 : 1	44
				6.30	189.0	178.9	166.6		
155a				6.31	187.4	173.6	166.4	1 : 1	33
155e					6.25	187.3	173.5		
	156c				6.23	187.0	173.6	166.7	0.9 : 1
156e					6.26	189.8	178.9	166.7	
	157a				6.23	187.0	173.6	166.6	1 : 1
158b						6.23	187.1	173.8	
	159c					6.34	187.1	173.8	166.7
159e					6.36	189.1	179.0	166.7	
	158b		6.14		186.5	173.5	166.7	1 : 1	43
159c				6.18	189.7	178.5	166.7		
	159c			6.23	187.0	173.6	166.7	0.9 : 1	44
159e				6.27	189.9	178.9	166.7		
	159e			6.24	186.9	173.6	166.7	0.9 : 1	47
159e				6.29	189.4	178.8	166.8		

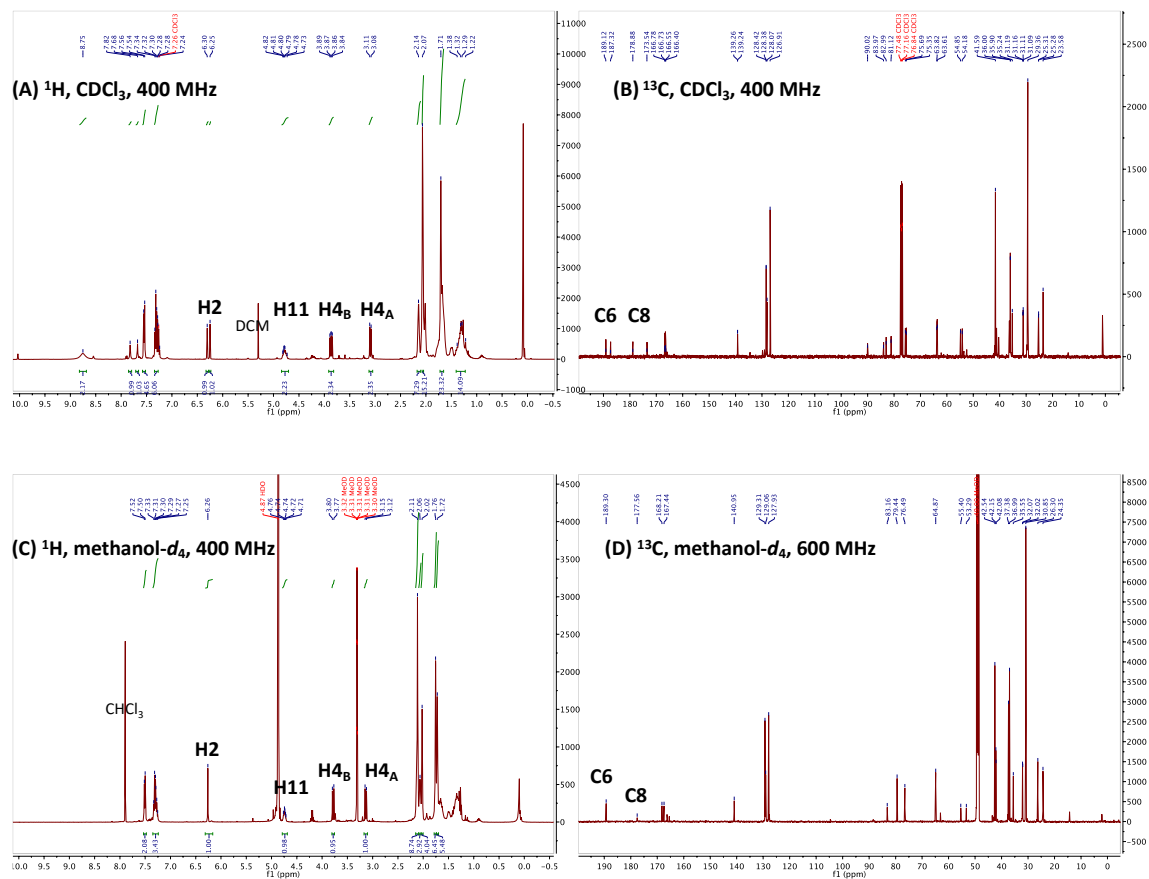
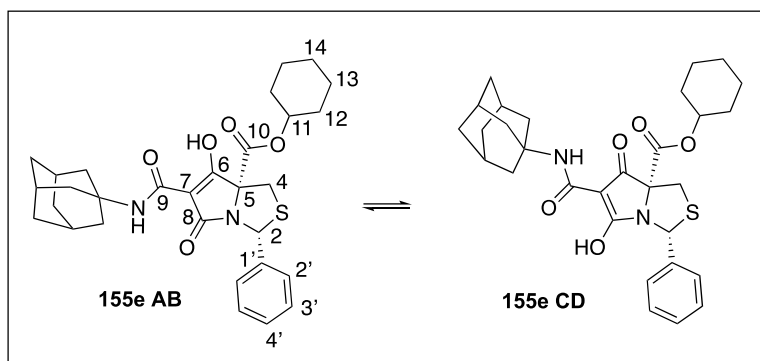
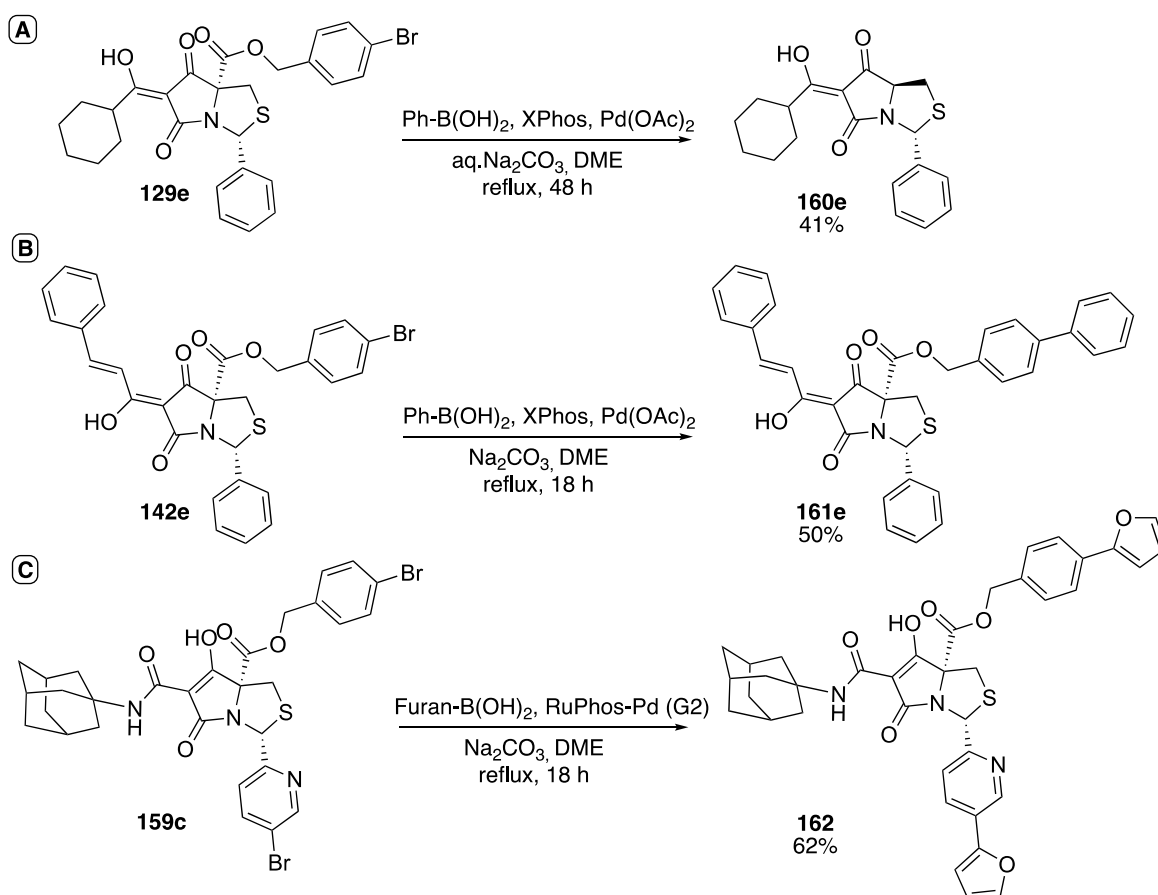


Figure 4.10 Comparison of NMR spectra of **155e** in CDCl_3 and methanol- d_4 showing equilibrium between two tautomeric forms.

4.3.3 Further possibility of late-stage functionalisation via Suzuki-Miyaura coupling

The presence of halogen atoms in C-5 esters and C-2 pyridyl rings allowed further functionalisation under the modified Suzuki-Miyaura coupling conditions.^{110,131,132} It would easily allow expansion of C-5 ester diversity via late-stage modifications, particularly when ester modification prior to Dieckmann cyclisation was limited due to stability of the resulting C-5 ester in a strained bicyclic tetramate system (page 130, *vide supra*). It would also allow fine-tuning of compound polarity as noted in Chapter 2 (section 2.6, page 65-66, *vide supra*). In order to demonstrate this possibility, tetramate ketones **129e**, **142e** and tetramate carboxamide **159c** were taken for Suzuki-coupling (Scheme 4.15).

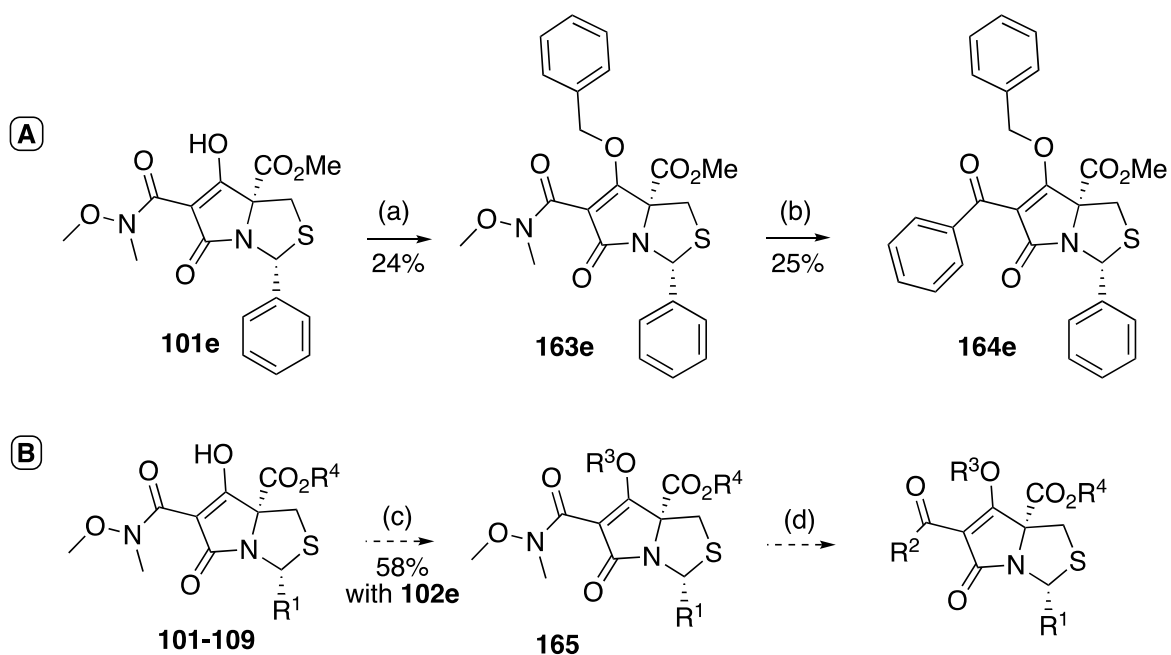


Scheme 4.15 Elaboration of C-5 esters and C-2 substituents through Suzuki-Miyaura coupling reactions.

The aqueous media used in the original reaction conditions unfortunately caused decarboxylation at C-5, probably followed from a base-catalysed hydrolysis of the C-5 ester (Scheme 4.15 A). Successful coupling was achieved by changing to dry solvent conditions (Scheme 4.15 B). The yield of coupling could be further improved with the use of RuPhos-Pd (G2) catalyst instead of the original XPhos/Pd(OAc)₂ catalytic system (Scheme 4.15 C).

4.3.4 Suggestion for further functionalisation on tetramate Weinreb amides

The more upfield C-6 carbonyl chemical shifts consistently observed in tetramate Weinreb amides as compared with tetramate carboxamides and ketones suggested a more shielded C-6 enol in tetramate Weinreb amides. Indeed, while previous attempts of direct alkylation with alkyl halides under basic conditions were completely unsuccessful with tetramate esters, tetramate carboxamides and tetramate ketones, some transformation was observed with tetramate Weinreb amide **101e**, with the desired *O*-alkylated product being isolated in 24% yield (Scheme 4.16 A). The apparent enhancement of nucleophilicity of the C-6 enol could be attributed to delocalisation of the nitrogen lone pair with the carbonyl group in Weinreb amide, opposing the delocalisation of C-6 enol O-lone pair. The enhanced nucleophilicity of the C-6 enol group can be utilised to incorporate a sugar derivative at C-6 under specific glycosylation conditions (Scheme 4.16 B),¹⁶⁶ which follows the concept of prodrug design introduced in Chapter 3. Under the Schmidt glycosylation conditions,^{166,196} a model reaction of *O*-glycosylation of Weinreb amide **102e** was achieved with 58% yield. However, the selectivity between α/β anomers and stability of the glycosyl bond when subjected to further functionalisation required further investigation.



Scheme 4.16 Towards incorporation of C-6 sugar derivative. (A) Attempted C-6 functionalisation of Tetramate Weinreb amide **101e**; (B) Proposed synthesis towards incorporation of a C-6 sugar derivative in tetramate analogues. *Reagents and conditions:* (a) benzyl bromide, powdered K_2CO_3 , catalytic benzyltributylammonium chloride, $CHCl_3$, r.t., o.n.; (b) R^2MgBr , dry THF, $-15\text{ }^\circ C$, 1 h; (c) 2,3,4,6-*tetra-O*-acetyl- β -galactopyranosyl (R^3) trichloroacetimidate, TMSOTf, dry DCM, $0\text{ }^\circ C$, 1 h; (d) Grignard reactions with R^2MgBr , dry THF, $-15\text{ }^\circ C$ or aminolysis with respective amines, THF/toluene, reflux followed by hydroxyl deprotection on the sugar.

4.4 Metal chelating properties of the synthesised tetramates

C-7 Acylated tetramate ketone analogues **112-142** were metal-chelating not surprisingly, with their chelating abilities influenced by the bulkiness of both C-5 esters and C-9 ketone pendants. With abundance of Mg^{2+} ions after the Grignard reactions, no discernible product peaks could be detected in the crude mixture of **130e** prior to acidic work-up (Figure 4.11 A). The crude material was washed with 0.5 M HCl to remove chelated metals (Figure 4.11 B). When subjected to flash column chromatography, the tetramate ketone analogue again bound with metal impurities from silica and/or sand, giving broad product peaks in the 1H NMR spectrum (Figure 4.11 C). Tetramate ketone analogues were routinely washed with 0.5 M HCl post-column to render them metal-free (Figure 4.11 D). Similar metal chelating behaviours were observed across the C-9 ethyl, phenethyl and styrenyl tetramate ketone series. With bulkier C-5 esters such as in the case of **135e**, there was an improvement in the resolution of peaks post-column as compared to tetramate analogues with C-5 methyl esters (Figure 4.11 E), supporting the hypothesis that steric hindrance posed by neighbouring C-5 esters reduced metal chelation. C-9 Cyclohexyl ketones **123-127** demonstrated a similar broadness of peaks post-column before acid wash. Interestingly, in tetramate analogues **128e** and **129e** with bulkier C-5 benzyl or substituted benzyl esters, comparably sharp product peaks were detected on 1H NMR spectroscopic examination of the crude and post-column samples prior to acid wash (Figure 4.12), suggesting further reduced metal binding abilities in these compounds. Comparison with tetramate ketones with the same C-5 ester also indicated the role played by the C-9 ketone pendant group in reducing metal binding. The presence of C-2 pyridyl rings in **128c** and **141c** again broadened the post-column product peaks before acid wash, suggesting that the C-2 pyridyl rings could also participate in extended metal

chelation (Figure 4.13 A). As previously reported tetramate ketones with C-5 methyl ester required 2M HCl solution to wash out metal ions,¹¹² the fact that **128c** and **141c** could be washed free from metal ions with a much weaker 5% citric acid solution to give well-resolved and sharp ¹H NMR peaks suggested much reduced metal chelation resulting from the presence of a bulky C-5 ester in these compounds (Figure 4.13 B).

In order to investigate the types of metal ions potentially involved in chelation, metal-free tetramate ketone analogue **130e** was deliberately washed with 1M aq. Ca(NO₃)₂ solution (Figure 4.14 A) and aq. 1M FeSO₄ solution (Figure 4.14 B). The extent of peak broadening suggested a higher affinity of metal chelation for Ca²⁺ than Fe²⁺. The peak broadening by washing metal-free samples with aq. Ca(NO₃)₂ solution was also observed previously in tetramate ketone analogues with C-2 *t*-butyl substituents (Scheme 4.8 B, page 125, *vide supra*).¹¹² This observation in tetramate ketones was in contrast to tetramate esters and carboxamides, which demonstrated a preference for Fe²⁺ with significant peak broadening when washed with the same strength of metal solution (Figure 2.16, page 63, *vide supra*). This difference again highlighted the structural dependency of metal chelation in tetramate derivatives.

In addition, analysis by SFC-MS for post-column sample of **133e** prior to acid wash indicated the presence of *m/z* peaks that could be attributed to [M+Ca]²⁺ and [M+Mg]²⁺ but not [M+Fe]²⁺ or [M+Fe]³⁺ (Figure 4.15), suggesting the relative abundance of Ca and Mg ions in silica and the possibility of metal chelation of tetramate ketone analogues with these divalent metal ions. The presence of Ca ions in post-column metal-chelated tetramate ketone analogues was also previously indicated by XPS analysis.¹¹²

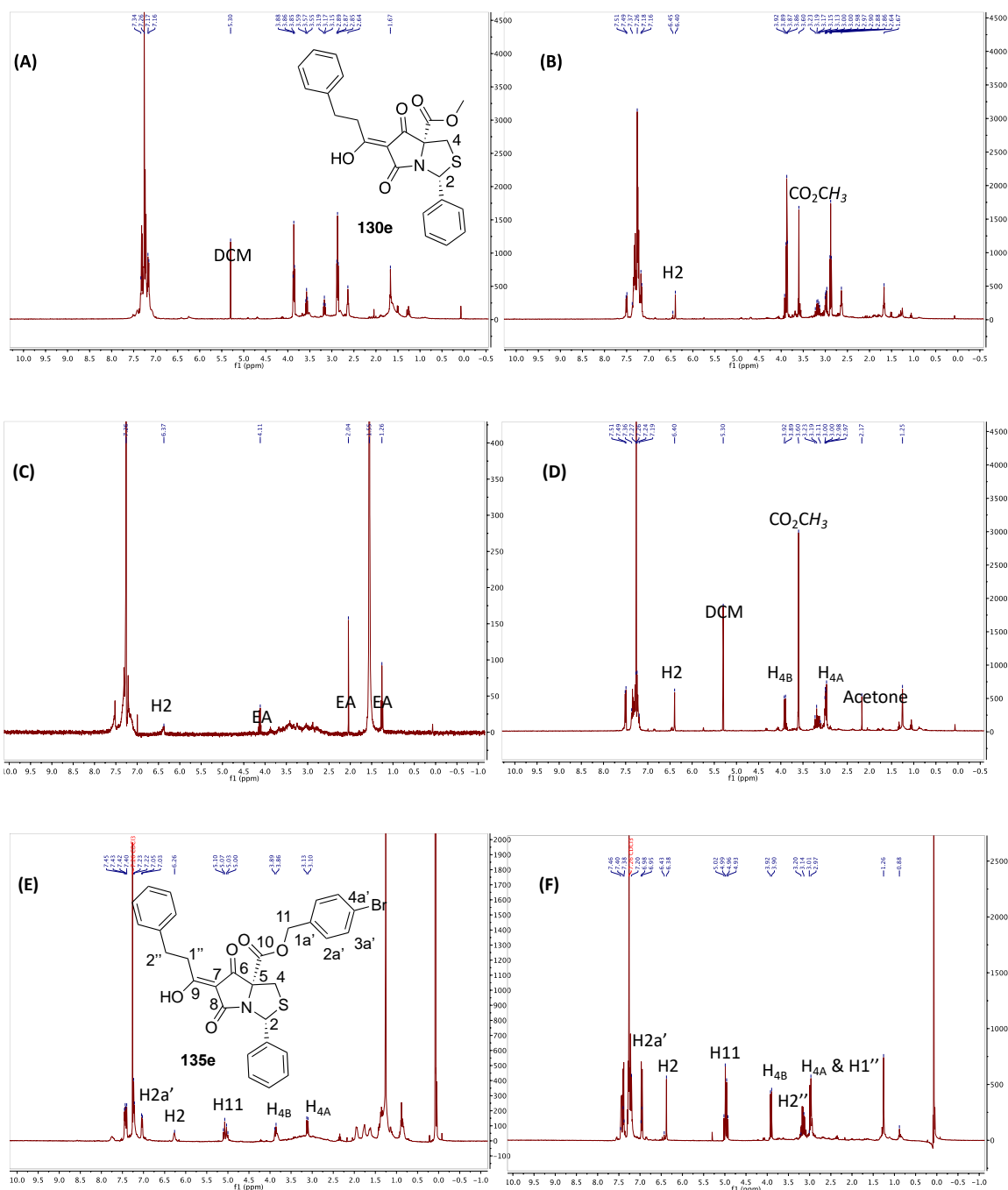


Figure 4.11 ^1H NMR (CDCl₃, 400 MHz) spectra indicating metal-chelation abilities of tetramate ketones **130e** and **135e**. (A) **130e** reaction crude mixture quenched with sat. aq. NH₄Cl and extracted with EA; (B) **130e** reaction crude mixture washed with 0.5 M HCl and extracted with EA; (C) **130e** purified with column, before acid wash; (D) **130e** purified with column, after 0.5 M HCl acid wash; (E) **135e** purified with column, before acid wash; (F) **135e** purified with column, after 0.5 M HCl acid wash.

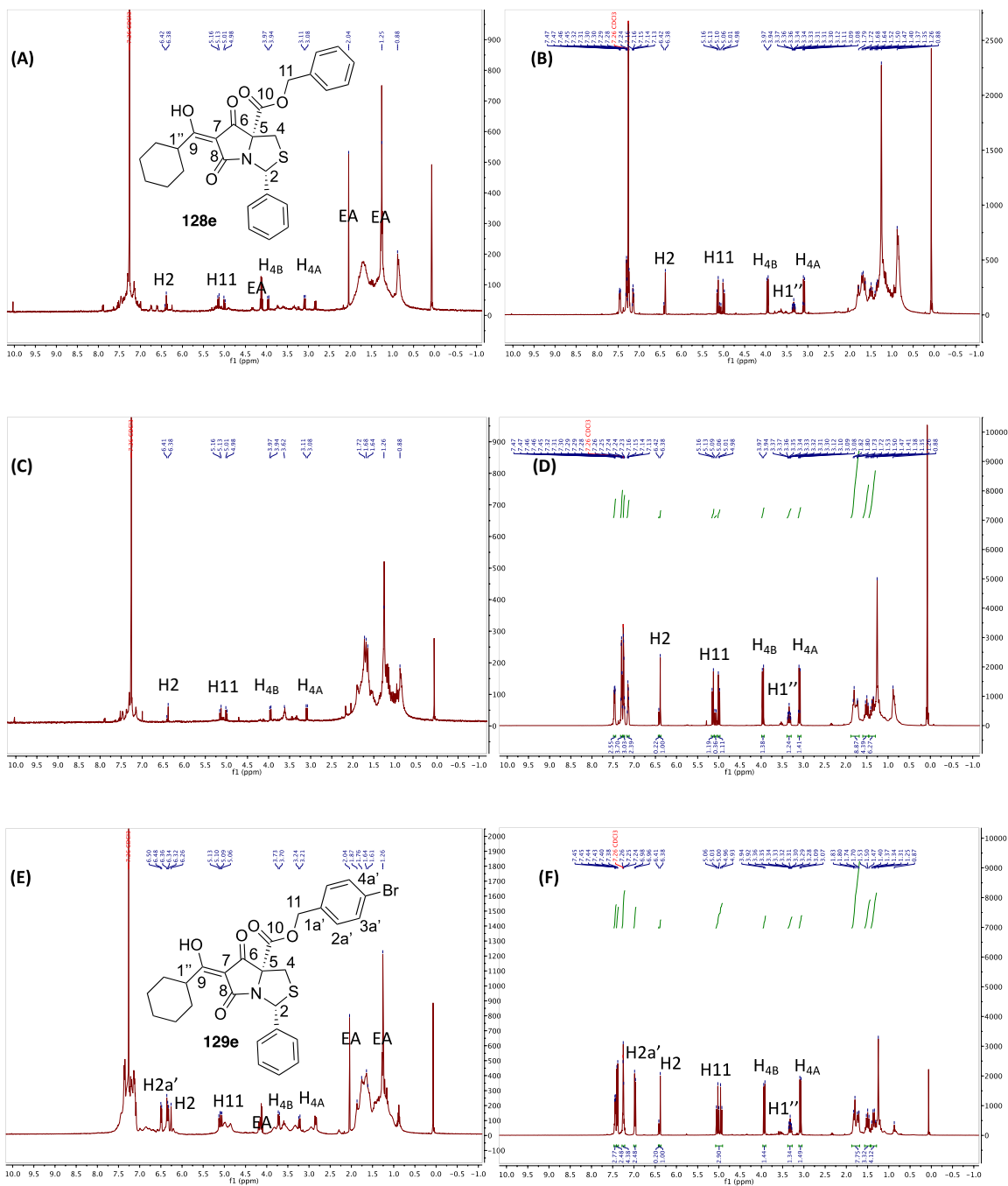


Figure 4.12 ^1H NMR (CDCl_3 , 400 MHz) spectra indicating reduced metal chelating abilities of tetramate ketones **128e** and **129e**. (A) **128e** reaction crude mixture quenched with sat. aq. NH_4Cl and extracted with EA; (B) **128e** crude washed with 0.5 M HCl and extracted with EA; (C) **128e** purified with column, before acid wash; (D) **128e** purified with column, with 0.5 M HCl acid wash; (E) **129e** purified with column, before acid wash; (F) **129e** purified with column, with 0.5 M HCl acid wash.

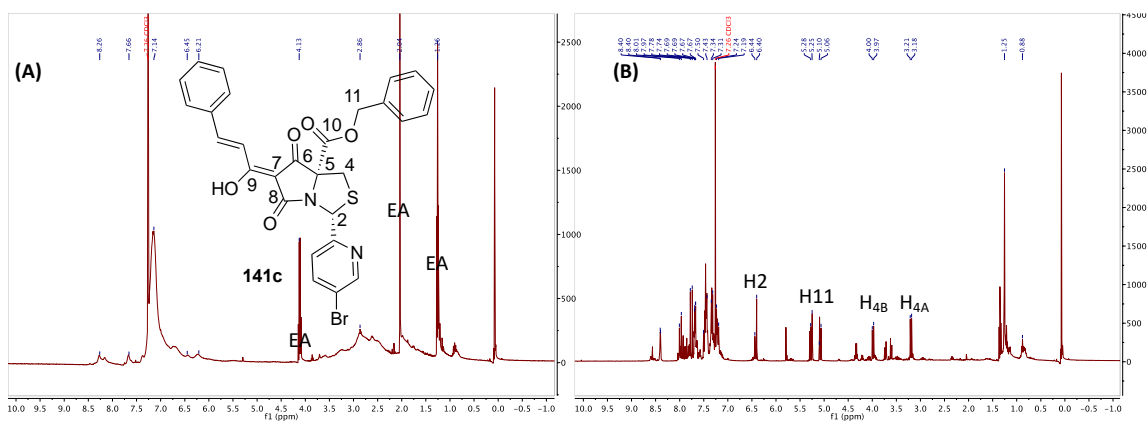


Figure 4.13 ^1H NMR (CDCl_3 , 400 MHz) spectra for metal chelating abilities of tetramate ketone **141c** with a C-2 pyridyl ring. (A) **141c** purified with column, before acid wash; (B) **141c** purified with column, with 5% citric acid wash.

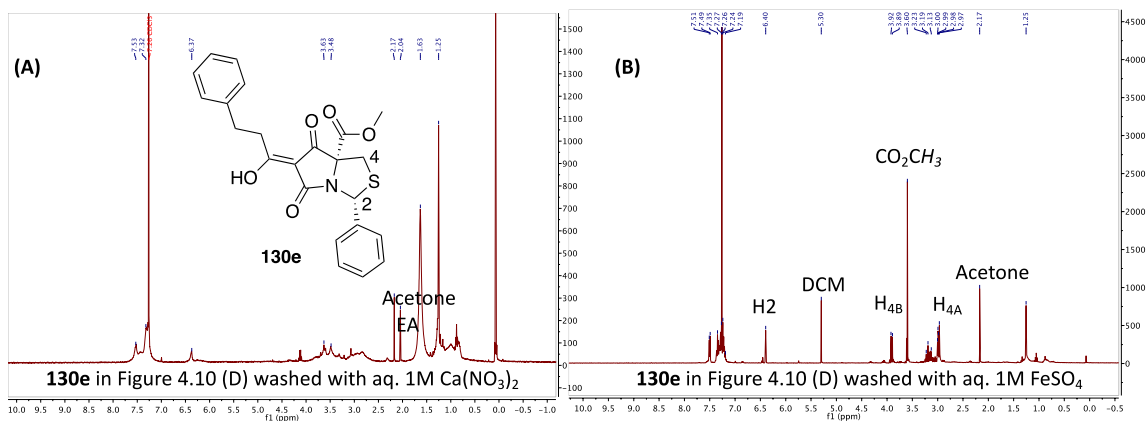


Figure 4.14 ^1H NMR (CDCl_3 , 400 MHz) spectra indicating a preference of **130e** for binding with specific metal ions. (A) Metal-free **130e** washed with aq. 1M $\text{Ca}(\text{NO}_3)_2$ solution; (B) Metal-free **130e** washed with aq. 1M FeSO_4 solution.

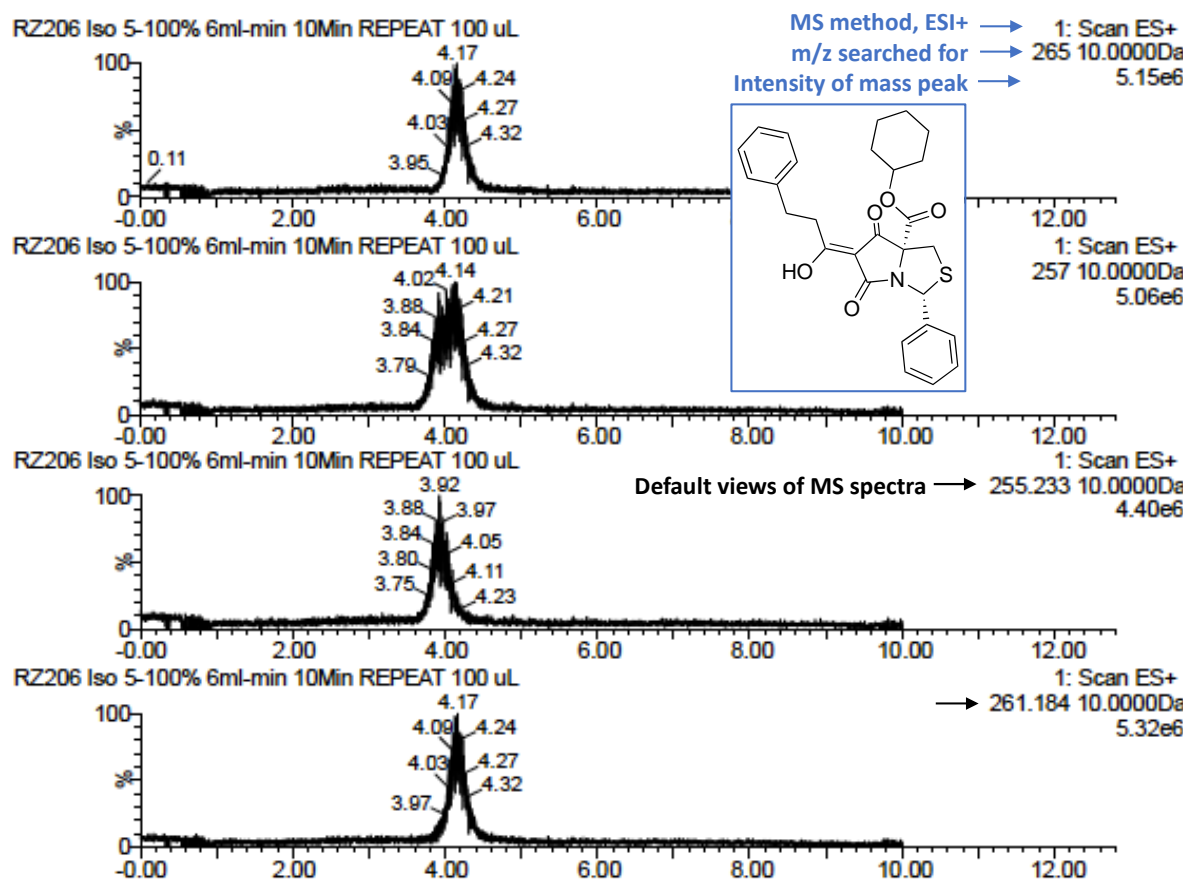


Figure 4.15 SFC-MS spectra for post-column metal chelated **133e** (SFC-MS, Oxford Suzhou Centre for Advanced Research, OSCAR). Exact mass $M = 491.18$; $[M+Ca]^{2+}$ $m/z = 265$; $[M+Mg]^{2+}$ $m/z = 257$. The peak intensity for searched m/z had to be comparable to those present in the default view in order to consider it as detected.

Tetramate carboxamides **145-159** with C-5 esters present were also found to be metal-chelating, with broadness of product peaks post-column but prior to acid wash (Figure 4.16 A). Tetramate carboxamides were also routinely washed with 0.5 M HCl solution to wash off metal ions post-column (Figure 4.16 B). 5% Citric acid solution was used instead if pyridyl rings were present. Comparing tetramate carboxamide **157a** with a bulky C-5 nitro-benzyl ester and previously synthesised carboxamide **30a** with no C-5 ester, 5% citric acid wash could result in a better-resolved and sharp 1H NMR spectrum in **157a**

while the H-2 and H-5 signals of **30a** still remained relatively broad after acidic wash (Figure 4.16 C and D). The relative extent of metal binding with increasing bulkiness of C-5 ester was not discernible by visual comparison of ^1H NMR spectra. With an NH linker, the bulkiness of C-9 amide pendant probably played a less important role than C-9 ketone pendant in reducing metal chelation by sterically influencing the nearby C-9 carbonyl. Width at half height of the H-2 signal was measured for each post-column sample as a quantitative indicator of metal binding and details are given in Chapter 5.

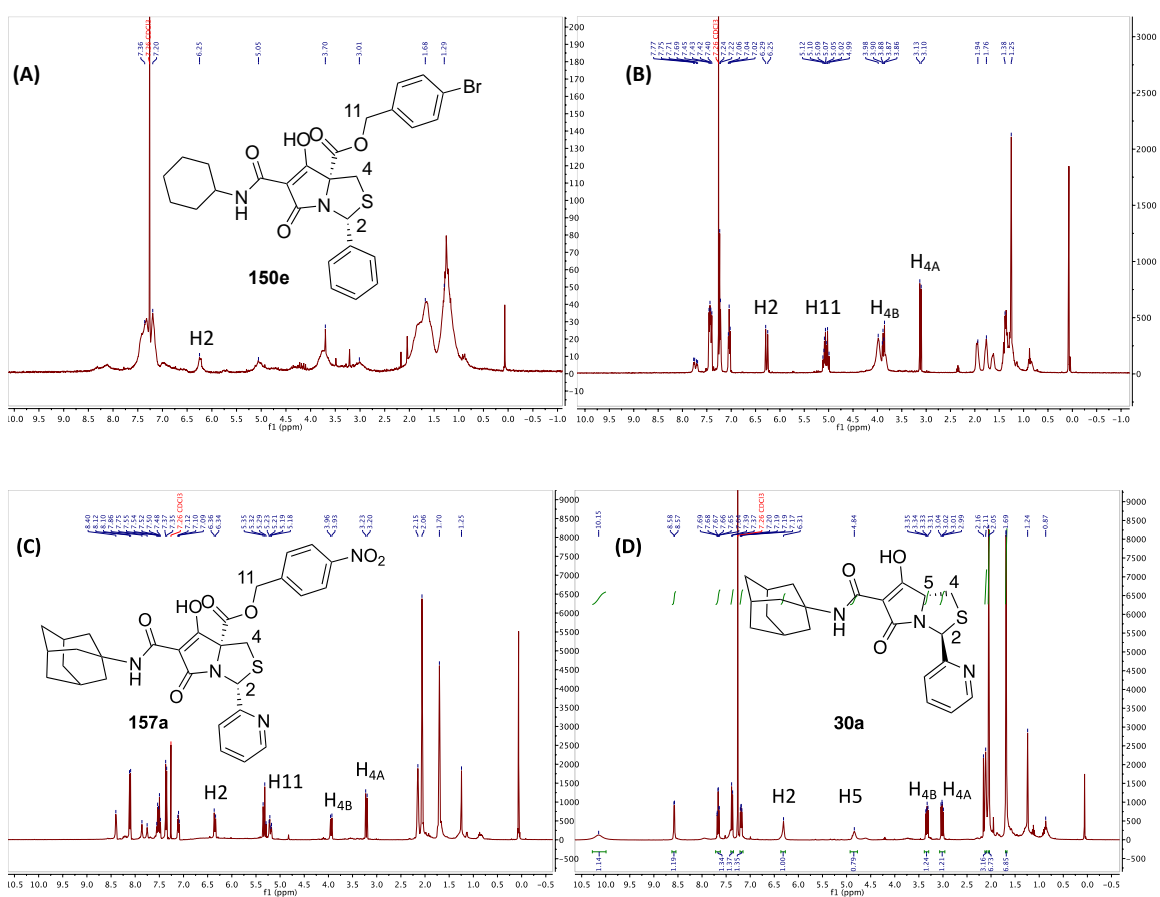
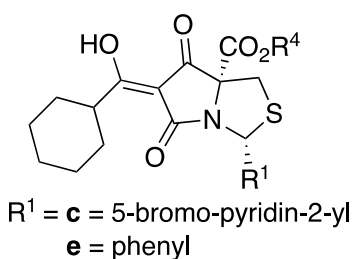


Figure 4.16 ^1H NMR (CDCl_3 , 400 MHz) spectra indicating metal chelating abilities of tetramate carboxamides **150e** and **17e**. (A) **150e** purified with column, before acid wash; (B) **150e** purified with column, with 0.5 M HCl acid wash; (C) **17e** purified with column, with 5% citric acid wash; (D) Previously synthesised tetramate carboxamide **30a** with no C-5 ester present, purified with column with 5% citric acid wash.

4.5 Biological evaluation

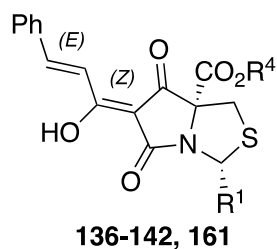
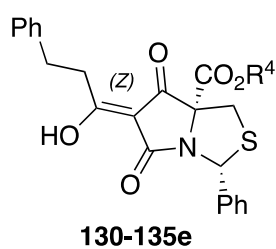
Tetramate ketones **111-144**, **160-161** and carboxamides **145-159**, **162** were tested for their antibacterial activities against Gram-positive MRSA, *Streptomyces* and Gram-negative *E. coli* (Table 4.10). No activity against *E. coli* was observed. Tetramate ketone analogues **112-122e** with C-9 ethyl or phenyl ketone pendants were not active while **123-142** with C-9 cyclohexyl, phenethyl and styrenyl ketone pendants were active against Gram-positive MRSA and *Streptomyces*. Tetramate carboxamides **145-159** with C-9 cyclohexyl or adamantyl amide pendants also demonstrated activities against Gram-positive bacteria. A key initial observation was that modifications at C-5 esters did not result in activity loss, with bulkier C-5 esters apparently enhancing the antibacterial potency against MRSA. A more detailed analysis of structure-activity relationship is described in Chapter 5.

Table 4.10 Biologically active cysteine-derived tetramate analogues.



	R ⁴ =	MIC (μg/ml) against		MW	clogP ^a	PSA ^a /Å ²
		MRSA	<i>Streptomyces</i>			
123e		15.6	7.81	401	2.82	83.9
125e		7.81	3.91	428	3.56	83.9
126e		7.81	7.81	430	3.58	83.9
127e		3.91	3.91	469	4.41	83.9
128c		15.6	15.6	557	4.36	96.8
128e		3.91	3.91	478	4.44	83.9
129e		1.95	0.49	556	5.23	83.9

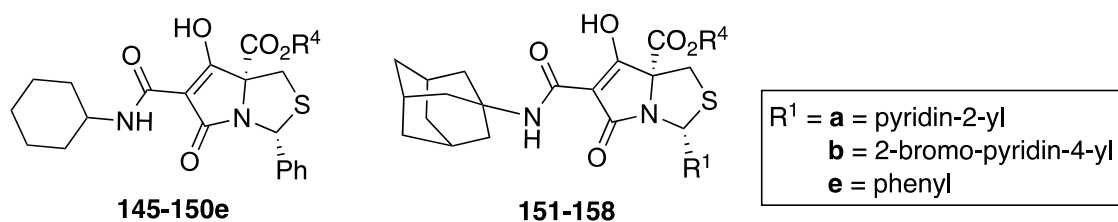
Table 4.10 Continued Biologically active cysteine-derived tetramate analogues.



R¹ = **c** = 5-bromo-pyridin-2-yl
e = phenyl

	R ⁴ =	MIC (μg/ml) against		MW	clogP ^a	PSA ^a /Å ²
		MRSA	<i>Streptomyces</i>			
130e		15.6	Not tested	423	3.35	83.9
131e		0.98	Not tested	450	4.09	83.9
132e		3.91	Not tested	452	4.11	83.9
133e		0.98	Not tested	492	4.94	83.9
134e		3.91	Not tested	500	5.13	83.9
135e		15.6	7.81	578	5.92	83.9
136e		15.6	Not tested	421	3.48	83.9
138e		7.81	7.81	448	4.22	83.9
139e		1.95	7.81	450	4.24	83.9
140e		1.95	1.95	490	5.07	83.9
141c		15.6	15.6	577	5.01	96.8
141e		3.91	7.81	498	5.26	83.9
142e		0.98	Not tested	576	6.05	83.9
161e		3.91	3.91	574	6.94	83.9

Table 4.10 Continued Biologically active cysteine-derived tetramate analogues.



	R ⁴ =	MIC (µg/ml) against		MW	clogP ^a	PSA ^a /Å ²
		MRSA	<i>Streptomyces</i>			
145e		62.5	62.5	416	1.21	95.9
146e		15.6	15.6	443	1.95	95.9
147e		15.6	15.6	445	1.96	95.9
148e		3.91	1.95	485	2.79	95.9
149e		15.6	15.6	493	2.99	95.9
150e		62.5	125	571	3.78	95.9
151e		15.6	7.81	469	1.34	95.9
152b		31.3	7.81	562	1.56	108.8
153e		15.6	15.6	495	2.08	95.9
154e		7.81	1.95	498	2.10	95.9
155a		7.81	7.81	538	1.89	108.8
155e		62.5	15.6	537	2.93	95.9
156e		62.5	31.3	545	3.12	95.9
157a		31.3	15.6	591	2.04	152.0
158b		62.5	31.3	670	2.95	152.0

[a] Relevant chemical properties of the active compounds were calculated using MarvinSketch 20.3.0, 2020, ChemAxon.

4.6 Summary

This chapter described the synthesis of cysteine-derived tetramate ketones and carboxamides with functionalisation at C-5 esters. The aim of such synthetic modifications was to decrease metal chelation by increasing bulkiness of the groups near the centre of metal binding. To achieve this goal, a library of L-cysteine esters was synthesised from acid-catalysed esterification of L-cystine in cyclohexane followed by selective reduction of the disulphide bond, with high yield and enantiopurity. The synthetic pathway then followed the methodology previously employed in the Moloney group with condensation with aldehydes and *N*-acylation. A key step was introduced in the synthetic route to incorporate a Weinreb amide to the *N*-acyl side chain of malonamides under Sucunza's acylation conditions, which allowed the subsequent Dieckmann cyclisation to proceed in the direction that retained the C-5 ester and C-7 Weinreb amide under modified cyclisation conditions with DBU. The resulting key intermediates, tetramate Weinreb amides, allowed diverse functionalisation to easily access C-7 ketones and carboxamides with modest yield. The target tetramate analogues were synthesised with a *cis* relationship between C-2 substituents and C-5 esters, as mixtures of tautomers in non-polar solvent such as CDCl₃. Qualitative comparison of post-column ¹H NMR spectra suggested evidence of reduced metal binding in tetramate analogues with bulkier C-5 esters and C-9 ketone pendants. Interestingly, modifications towards bulkier C-5 esters resulted in an enhancement of antibacterial activity against Gram-positive bacteria including MRSA, which indicated a potential of controlling metal chelation without compromising biological activity at C-5 position of the bicyclic tetramate system.

Chapter 5 Evaluation of biological properties

This final chapter describes the evaluation of antibacterial activity of the tetramate analogues whose synthesis was described in earlier chapters. Using broth dilution assay to determine MIC values, functionalised cysteine-derived tetramate carboxamides and ketones demonstrated selective inhibition against Gram-positive bacteria. Physicochemical properties are calculated for the tetramate analogues and correlated with their antibacterial activity to depict a characteristic chemical space. To reduce plasma protein binding, chemical modifications at C-2 can tolerate polar heterocycles to access more hydrophilic tetramate derivatives while preserving antibacterial activity. With analysis of the post-column broadening of ^1H NMR spectra, reduced metal binding is demonstrated with functionalisation at C-6 enol, C-9 carbonyl and C-5 esters, and some derivatives have exhibited good antibacterial activity, which serve as promising tetramate templates for further optimization.

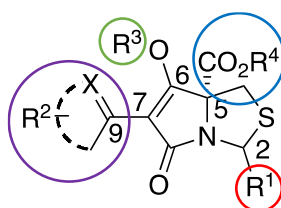


Figure 5.1 Chemical modifications of cysteine-derived tetramate analogues.

5.1 Introduction

As described in Chapter 1 (section 1.8, page 27-28, *vide supra*), this project started with the reported biological results of cysteine-derived tetramatic acid derivatives showing promising antibacterial activities, which unfortunately diminished when tested *in vitro* in

the presence of blood. We identified plasma protein binding and metal chelation as two blood-associated factors which might be implicated in this apparent reduction of antibacterial activities. Previous work in the Moloney group has demonstrated tetramate binding to human serum albumin (HSA) by an NMR study of a competitive displacement of L-tryptophan, which is a known site-specific HSA binder.¹¹⁰ The reduction of antibacterial activities due to tetramate binding to HSA was also indicated by a decrease in zone size of inhibition in hole-plate assays conducted in the presence of HSA.¹¹⁰ The metal chelating abilities of tetramate analogues have also been repeatedly demonstrated with post-column broadening of signals in their ¹H NMR spectra. When active tetramate carboxamides **28c**, **30a-d** (MIC ranging from 0.24 to 7.81 µg/ml) were tested in the presence of 0.01% Fe³⁺ solution, no activity was observed. When weakly active tetramate ketone **130e** (MIC = 15.6 µg/ml) was deliberately washed with 1M aq. Fe³⁺ solution and tested again, biological activity was lost completely. This suggested that metal binding of tetramate analogues could potentially lead to the apparent reduction of antibacterial activity. Hence, the main aims of this project were, firstly to reduce plasma protein binding and secondly to modulate metal chelation in cysteine-derived tetramate analogues while preserving good antibacterial activities.

Synthesised tetramate analogues were evaluated for their antibacterial activities by broth dilution assay to determine their MIC values (tested in collaboration with Oxford Antibiotic Group). This phenotypic whole-cell assay incorporates an assessment of all *in vitro* drug-related properties such as aqueous solubility, permeability through bacterial cell membrane, and accumulation within bacterial cells and targets interaction, to give an overall effectiveness of the tetramate analogues. Drug-likeness of active tetramate

analogues was further assessed by calculation of their physiochemical properties, which predict their plasma protein binding abilities, as well as oral bioavailability, aqueous solubility and permeability through bacterial cell wall/membrane structure (see section 5.2). The metal chelation properties of tetramate analogues were analysed separately by NMR study of post-column samples by a consideration of the broadening of signals and this is discussed in relation with their antibacterial activities (see section 5.3).

To tackle the problem of antibacterial resistance, an ideal antibacterial drug should be able to interact with multiple bacterial targets in different metabolic pathways essential for bacterial survival (Table 1.4, page 12, *vide supra*).³⁰ While biological targets for the synthesised tetramate analogues in this project remain to be elucidated, there were several reported biological mechanisms for similar tetramic acid derived systems. Naturally occurring kibdelomycin was reported to inhibit bacterial topoisomerase IV and DNA gyrase,^{42,44} streptolydigin was found to be capable of inhibiting bacterial RNAP,^{49,148} and signermycin B was found to exhibit inhibition of histidine kinase WaLK.⁴⁶ Previous work in the Moloney group also demonstrated that C-7 acyl tetramates possessed dual mechanisms of action against RNAP and UPPS while analogous tetramate carboxamides exhibited dual inhibition of Gram-positive *S. aureus* topoisomerase IV and RNAP.^{48,110} Topoisomerases and DNA gyrase are enzymes that relieve torsional strain of DNA double helices during DNA replication and transcription.¹⁹⁷ RNAP catalyses transcription.¹⁹⁸ Histidine kinase WaLK is found to be an indispensable phosphorylation enzyme in the signal transduction pathway for cell wall remodeling and division in Gram-positive *B. subtilis* and *S. aureus*.¹⁹⁹⁻²⁰¹ UPPS catalyses the production of UPP, which is an important component for the formation and growth of bacterial peptidoglycan cell wall.^{202,203} All of

these reported bacterial targets of tetramate-containing derivatives are essential for bacterial survival and known inhibitors of these targets were reported to be bactericidal.²⁰³⁻²⁰⁶ The structural properties of these active tetramic acids were taken into consideration during design of target compound libraries.

In addition, for antibacterial drugs, the ability to interact with multiple biological targets should be accompanied by a selectivity towards prokaryotic pathogenic systems and low off-target effects.³⁰ Among the reported bacterial targets, UPPS and histidine kinase WaLK are enzymes unique to bacterial systems for cell wall construction and metabolism;^{201,203} and bacterial RNAP possesses a highly conserved amino acid sequence, different from that of the eukaryotic RNAP, therefore allowing broad-spectrum antibacterial activities with therapeutic selectivity.^{207,208} Toxicity and prokaryotic selectivity of the synthesised tetramate analogues were briefly evaluated in the last section of this chapter (see section 5.4).

5.2 Physicochemical properties

The concept of an ideal antibacterial drug includes requirement for high oral availability, aqueous solubility and low protein plasma binding (Table 1.4, page 12, *vide supra*).³⁰ In order to evaluate the drug-likeness of the synthesised tetramate analogues, MW (Da), clogP, clogD_{7.4}, polar surface area (PSA/Å²) and relative PSA (as a % of molecular surface area, MSA/Å²) of the synthesised tetramate analogues were calculated and tabulated. For clogP and clogD_{7.4} predictions, the method for calculations was defined by Marvin 20.3.0, 2020, ChemAxon where the pool of fragments with assigned logP values is based on the data set of Viswanadhan et al.²⁰⁹ The list of parameters for all tested tetramate

derivatives is provided in the Appendix; only the active compounds are shown in Table 5.1. While a MIC of 16 - 32 µg/ml against tested pathogenic organism is considered a suitable minimum at a preliminary evaluation stage,³⁷ effective antibacterial activity of cysteine-derived tetramate analogues synthesised in this project was defined by an MIC against MRSA of < 8 µg/ml.³⁶

Table 5.1 Physicochemical properties of active tetramate analogues.^a

Cpd No.	MW /Da	clogP	clogD _{7.4}	PSA /Å ²	MSA /Å ²	% PSA	HBD	HBA	MIC against MRSA	
									µg/ml	µM
GM18	400.88	3.21	-0.40	69.64	482.40	14.4	2	3	3.91	9.75
GM22	410.53	1.78	-0.47	69.64	542.94	12.8	2	3	0.98	2.39
28c	438.34	1.41	-1.42	82.53	496.41	16.6	2	4	7.81	17.8
30a	411.52	0.75	-1.71	82.53	535.87	15.4	2	4	1.95	4.74
30b	490.42	1.66	-1.12	82.53	555.65	14.9	2	4	1.95	3.98
30c	490.42	1.54	-1.23	82.53	556.52	14.8	2	4	3.91	7.97
30d	445.96	1.36	-1.38	82.53	552.09	14.9	2	4	0.24	0.538
41e,7S	478.65	4.98	4.98	66.48	687.00	9.68	1	3	7.81	16.3
125e	427.52	3.56	0.88	83.91	572.86	14.6	1	4	7.81	18.3
126e	429.53	3.58	0.91	83.91	602.20	13.9	1	4	7.81	18.2
127e	469.50	4.41	1.72	83.91	662.98	12.7	1	4	3.91	8.33
128e	477.58	4.60	1.84	83.91	647.55	13.0	1	4	3.91	8.19
129e	556.47	5.39	2.60	83.91	668.03	12.6	1	4	1.95	3.50
131e	449.52	4.09	1.54	83.91	587.27	14.2	1	4	0.98	2.18
132e	451.54	4.11	1.58	83.91	616.53	13.6	1	4	3.91	8.66
133e	491.60	4.94	2.39	83.91	677.79	12.4	1	4	0.98	1.99
134e	499.59	5.13	2.51	83.91	662.21	12.7	1	4	3.91	7.83
138e	447.51	4.22	1.98	83.91	559.30	15.0	1	4	7.81	17.5
139e	449.52	4.24	2.02	83.91	588.30	14.3	1	4	1.95	4.33
140e	489.59	5.07	2.83	83.91	650.24	12.9	1	4	1.95	3.98
141e	497.57	5.26	2.95	83.91	634.90	13.2	1	4	3.91	7.86
142e	576.46	6.05	3.70	83.91	655.43	12.8	1	4	0.98	1.70
148e	484.61	2.79	-0.72	95.94	684.24	14.0	2	4	3.91	8.07
154e	496.62	2.10	-1.42	95.94	680.97	14.1	2	4	7.81	15.7
155a	537.68	1.89	-1.66	108.83	736.12	14.8	2	5	7.81	14.5
161e	573.66	6.94	4.61	83.91	745.11	11.3	1	4	3.91	6.82
Mean	476.07	3.75	1.34	82.53	621.96	13.4	NA	NA	NA	NA

[a] MW was calculated by ChemDraw 19.1.1.32; clogP, clogD_{7.4}, PSA, MSA, hydrogen bond donors (HBD) and acceptors (HBA) were calculated with Marvin (20.3.0), 2020, ChemAxon; % PSA was calculated by % PSA/MSA. (NA = Not applicable)

The ranges of physiochemical properties for active tetramate derivatives in this project are described as MSA of 482 - 746 Å², MW of 400 - 577 Da, clogP of 0.7 - 7.0, clogD_{7.4} of -1.7 - 5.0, PSA of 66 - 109 Å² and relative PSA of 9 - 17%. These physiochemical boundaries are comparable to antibacterial active tetramate carboxamides previously reported by the Moloney group (MSA of 530 - 600 Å², clogP of 1.2 - 2.5, clogD_{7.4} of -1.1 - 0, PSA of 65 - 80 Å² and relative PSA of 11 - 14%).⁴⁸ These properties also resemble those of clinically used antibacterial fluoroquinolones to be drug-like (MSA of 380 - 550 Å², clogP of 0.6 - 3.7, clogD_{7.4} of -2.7 - 0.5, PSA of 60 - 120 Å² and relative PSA of 13 - 24%).³⁶

The chemical space occupied by the active tetramate analogues was further delineated by plots of clogP, clogD_{7.4}, PSA and relative PSA against MW (Figure 5.2). It was interesting to note that for active tetramate analogues, their clogP and clogD_{7.4} values positively correlate with MW in an approximately linear relationship while relative PSA changes in the inverse direction to MW (Figure 5.2 A, B and D). Such trends in structure-activity relationships were previously noted for antibacterial active tetramate carboxamides.⁴⁸ Most of the active tetramate analogues possess a PSA of 82 - 84 Å² (Figure 5.2 C). The narrow ranges of PSA and relative PSA indicated the small variation allowed in polarity in maintaining antibacterial activity against MRSA.

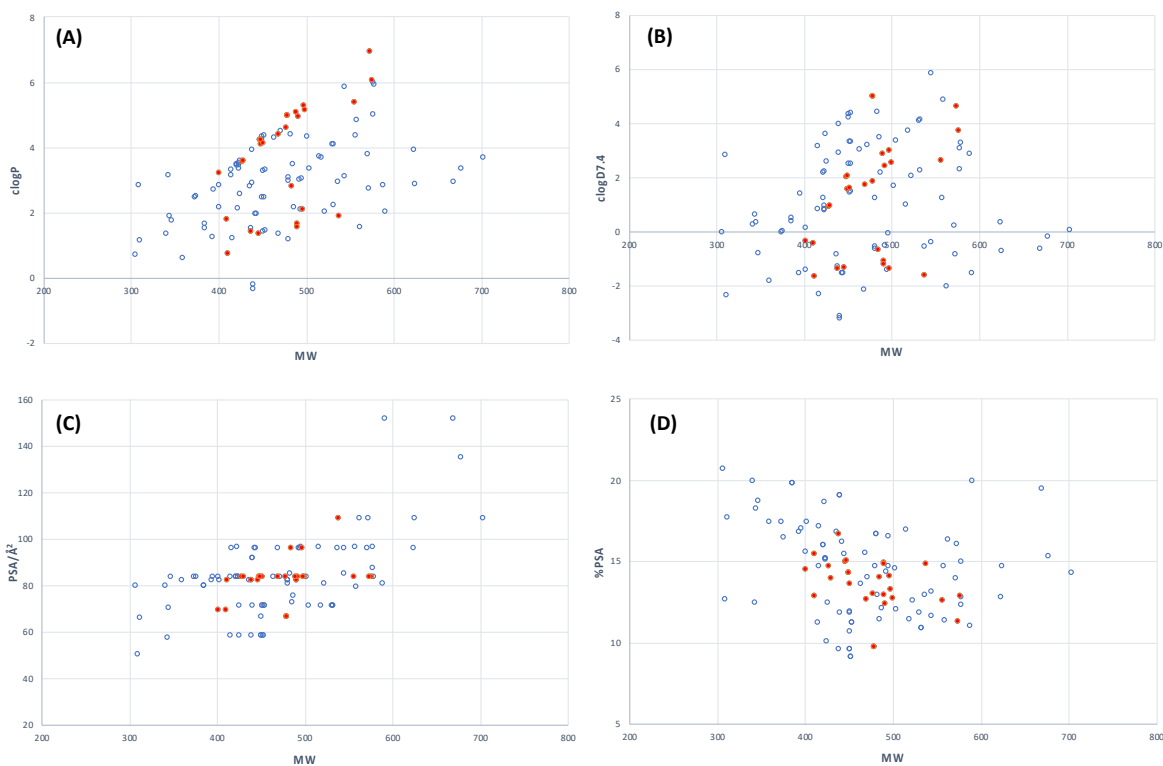


Figure 5.2 Plots of (A) clog P, (B) clogD_{7.4}, (C) PSA/Å² and (D) %PSA against MW/Da. Data representing the active tetramate analogues were highlighted in red.

In order to predict oral bioavailability, one commonly used rule is the Lipinski's rule of five, which states that an orally active drug has no more than one violation of the following physicochemical criteria, MW < 500 Da, clogP < 5, PSA < 140 Å², hydrogen bond donors (HBD) < 5 and hydrogen bond acceptors (HBA) < 10.³¹ While there are many extensions to the Lipinski's rule of five and many exceptions from the rule for known antibacterial drugs, most tetramate derivatives that were tested active fit comfortably within the limits. The compounds with parameters that deviate from the rule are highlighted in red (Table 5.1, page 164, *vide supra*). Especially for deviations in MW, it was noted that tetramate derivatives may be middle space compounds which can have good oral availability despite having relatively large MW.³⁸

One of the primary goals of this project was to reduce plasma protein binding by increasing compound hydrophilicity, which can be assessed indirectly by the calculated physiochemical properties. The main strategy to access hydrophilic tetramate analogues was to incorporate heterocyclic rings at C-2 (Figure 5.3). In the resulting tetramate derivatives **28c** and **30a-d**, improved hydrophilicity was indicated by an increase in PSA and relative PSA coupled with a decrease in clogP and clog_{7.4} values as compared to GM18 and GM22 previously synthesised in the Moloney group (Table 5.1, page 164, *vide supra*).

Introduction of C-5 esters in tetramate carboxamides and ketones was inevitably accompanied by an increase in PSA (compounds **125-155**, Table 5.1, page 164, *vide supra*). These tetramate analogues with C-5 esters did not seem to tolerate well the insertion of an additional polar substituent at C-2 (Figure 5.3). For example, tetramate ketone **128c** with a C-5 benzyl ester and C-2 bromopyridyl ring was only weakly active with an MIC of 15.6 µg/ml against MRSA while its C-2 phenyl analogue **128e** was more than 3 times active with an MIC value of 3.91 µg/ml (Figure 5.3). Similar reduction in activity was observed with **141c** as compared to **141e** (Figure 5.3). Tetramate ketones **124b**, **137b** and tetramate carboxamides **149c**, **156c** and **162**, all bearing C-2 pyridyl rings, were tested negative against MRSA, although the C-2 phenyl analogues **149e** and **156e** were only weakly active in the first place with MIC values of 15.6 and 62.5 µg/ml respectively (Figure 5.3). It was interestingly to note that tetramate carboxamide **155a** with C-2 pyridin-2-yl ring and C-5 cyclohexyl ester possessed promising antibacterial activity of MIC against MRSA at 7.81 µg/ml (Figure 5.3). This tetramate analogue **155a** also demonstrated marked improvement in polarity as indicated by its clogD_{7.4}, PSA and relative PSA values as compared to previously synthesised GM18 and GM22 (Table 5.1, page 164, *vide supra*).

Introduction of polar heterocycles at C-9 amide/ketone pendants in tetramate analogues **29**, **35**, **143e** and **144e** consistently resulted in a loss of antibacterial activity (Figure 5.3). Unfunctionalised tetramate esters **25a-d** were inactive. Tetramate carboxamides **27a-d** and ketones **112-121** with phenyl ring or short ethyl chain at C-9 pendant were all inactive. These indicated that a bulky hydrophobic core at C-9 pendant was essential to confer activity against MRSA. It was suggested that for the tetramate template shown in Figure 5.1 (page 160, *vide supra*), C-2 was the most suitable position for polarity adjustment to reduce plasma protein binding while preserving good antibacterial activity.

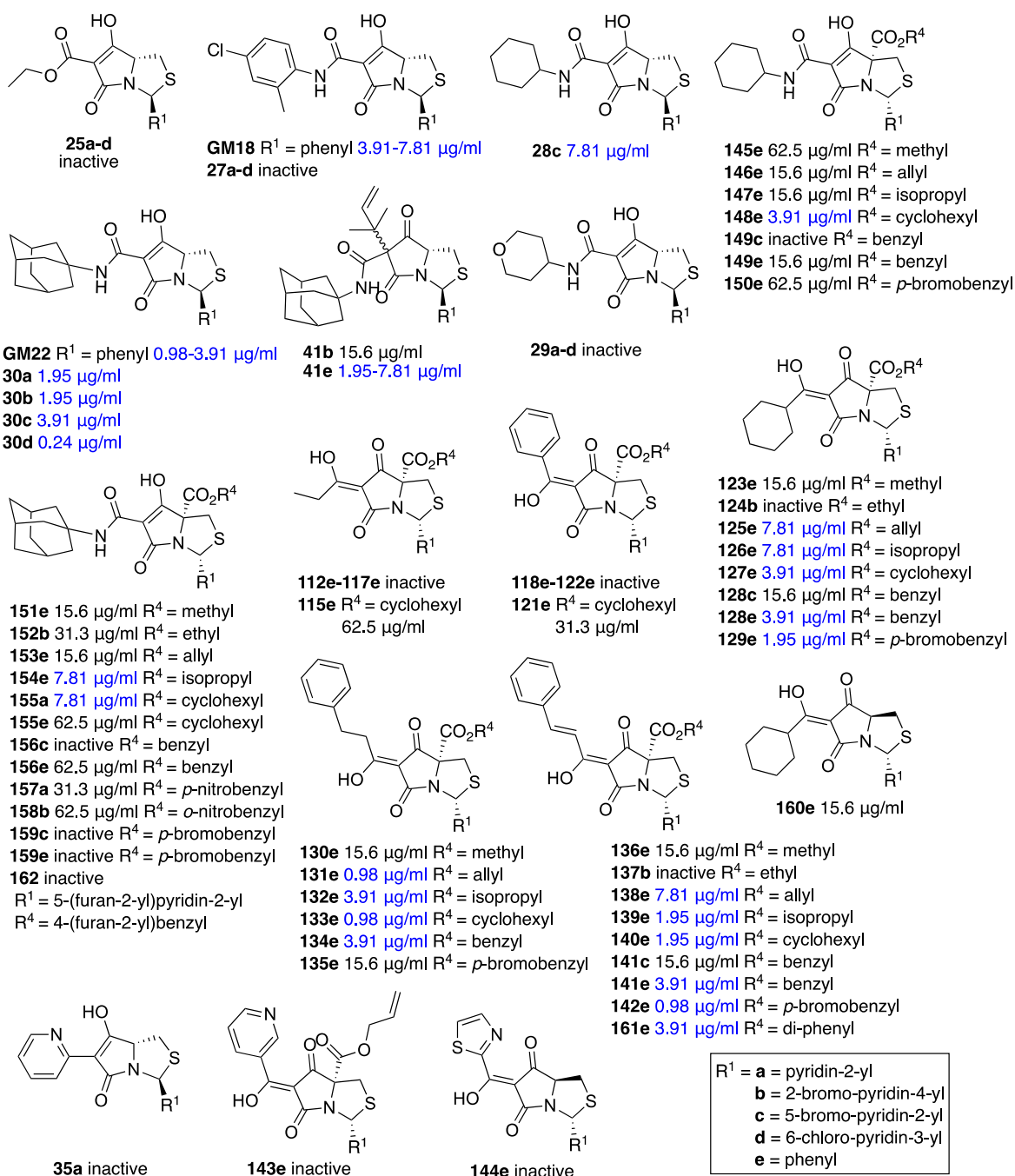


Figure 5.3 Structural analysis of the synthesised tetramate analogues (MIC values against MRSA, active ones with MIC < 8 µg/ml are highlight in blue).

5.2.1 Selective Gram-positive activity

While a subset of the synthesised tetramate analogues demonstrated good antibacterial activity against Gram-positive strains of bacteria including MRSA, no activity in Gram-negative *E. coli* was observed. While this was disappointing, it was nevertheless in accordance with previous observations that Gram-positive and Gram-negative activities have different requirements of physiochemical properties.³⁶ Gram-negative bacteria contain an additional outer cell membrane as compared to Gram-positive bacteria.²¹⁰ This outer cell membrane acts a barrier for molecule penetration; and it contains water-filled protein porins responsible for the uptake of most antibacterial drugs.²¹⁰ Therefore, compounds reported with Gram-negative activities need generally to be more polar to penetrate through these hydrophilic protein porins.³⁶ The size of the protein channels also poses an upper limit to MW of the compounds (generally MW < 600 Da).²¹¹ In addition, it was previously reported in the Moloney group that larger and more lipophilic C-7 acyltetramates with MW > 620 Da, clogP > 3, clogD_{7.4} > 1, relative PSA < 12% were more easily transported by efflux pumps in Gram-negative *H. influenza*.⁴⁸ Among tetramate analogues that were tested active against MRSA, while none of their MW exceeds 620 Da and most of their relative PSA were > 12%, majority of them have clogP > 3 and clogD_{7.4} > 1 (Table 5.1, page 164, *vide supra*), which increased their likelihood of being transported out by efflux pumps in Gram-negative bacteria. The trend of differential requirements between Gram-positive and Gram-negative activities was also noted by O'Shea and Moser where they reported the average physiochemical properties for selective Gram-negative activity as MW = 414, clogP = - 0.1, clogD_{7.4} = - 2.8 and relative PSA = 42% and for selective Gram-positive activity as MW = 813, clogP = 2.1,

$\text{clogD}_{7.4} = -0.2$ and relative PSA = 30%.³⁶ The physiochemical properties set the boundary for good permeability through the respective bacterial cell wall and membrane structure.

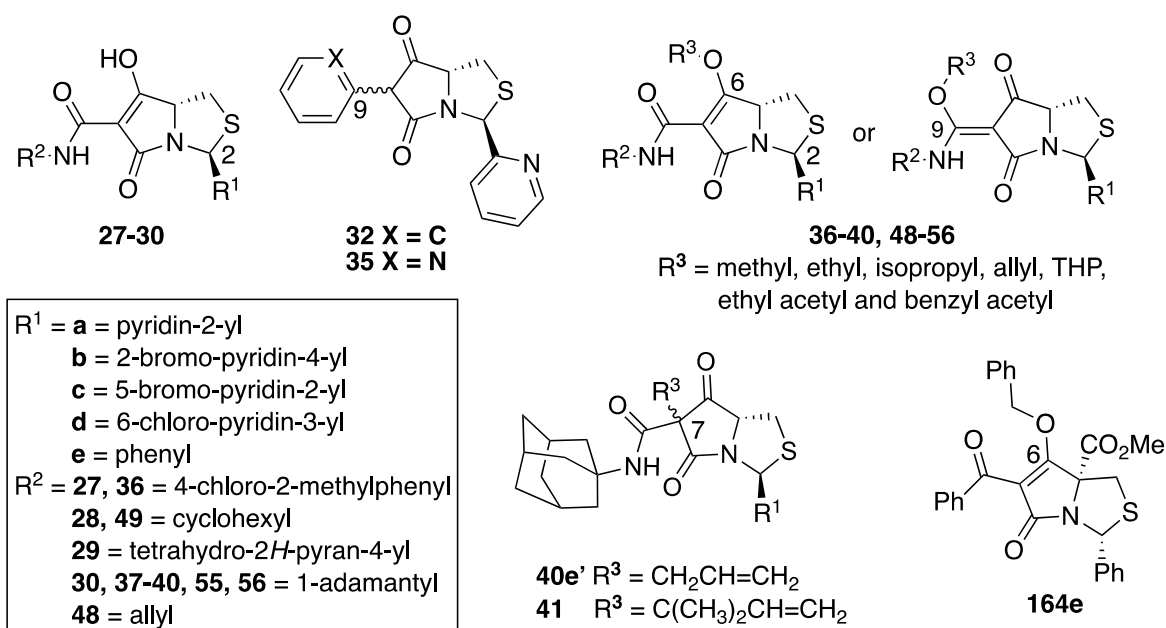
Most tetramate-containing natural products exhibited selective activity against Gram-positive bacteria while only a few such as epicoccarine A, zopfiellamide A and kibdelomycin have been reported with antibacterial activities against Gram-negative bacteria and mycobacteria which have more complex cell wall and membrane structures (Table 1.5, page 15, *vide supra*).³⁹⁻⁴⁶ This probably suggests that tetramate-based derivatives generally occupy a chemical space more in accordance with the requirement for Gram-positive activities. The average values of physiochemical properties for the active tetramate analogues are MW = 476, $\text{clogP} = 3.8$, $\text{clogD}_{7.4} = 1.3$ and relative PSA = 13% (Table 5.1, page 164, *vide supra*), which fit better into the chemical space as described by O'Shea and Moser for selective Gram-positive activities.³⁶

5.3 Metal chelation properties

The other primary goal of this project was to modulate metal binding properties of the tetramate analogues. Tetramate-containing natural products and their derivatives were well-known for their metal-binding properties, which are often correlated with their biological activities.^{47,59} The synthesised tetramate analogues also exhibited structure-dependent metal chelation, consistently demonstrated by peak broadening of ^1H NMR spectra after column chromatography. Qualitative visual comparison of the post-column ^1H NMR spectra was detailed in section 2.4 (page 62-63), section 3.4 (page 103-104) and section 4.4 (page 149-155, *vide supra*). Evidence from deliberate washing with specific metal ion solutions and SFC-MS analysis also showed that tetramate esters and

carboxamides bind strongly with Fe^{2+} while tetramate ketones bind preferentially with Ca^{2+} and Mg^{2+} (section 2.4, page 62-63; section 4.4, page 153-154, *vide supra*). In order to quantify the metal chelation property, width at half height ($W_{1/2}$) values for the H-2 signals in post-column ^1H NMR spectra were calculated as estimates of the extent of peak broadening as a measure of the extent of metal binding. Series of tetramate analogues were selected and their calculated data were tabulated (Table 5.2 - 5.4). It was noted that the H-2 signals of metal-chelated tetramates hardly assume perfect Lorentzian shape, but their widths were nevertheless used as a rough estimate for the extent of peak broadening. In cases where two sets of H-2 signals were present in the NMR spectrum of a single compound due to tautomeric behaviour, the major peak was taken for calculation.

Table 5.2 Post-column widths at half height/Hz for H-2 signals of tetramate analogues.^a



Cpd No.	W _{1/2} /Hz	Cpd No.	W _{1/2} /Hz	Cpd No.	W _{1/2} /Hz	Cpd No.	W _{1/2} /Hz
27a	35.9	32	3.0	39c-1	2.0	41e	1.8
27d	15.6	35	5.3	39c-2	1.9	48c	1.6
28a	15.4	36e	2.4	39e	2.1	49c	2.4
28b	36.4	37a	1.8	39e	2.2	50c	1.7
28c	13.7	37c	1.7	40a-1	2.8	54e	2.4
28d	48.5	37e	2.2	40a-2	2.2	55e	2.1
29b	16.4	38a	1.9	40c	1.7	56e	2.2
30a	25.2	38c	1.8	40e	2.2	164e	2.0
30b	16.1	38e	2.2	40e	2.1		
30c	35.2	39a-1	1.9	40e'	2.0		
30d	24.1	39a-2	2.3	41b	2.1		
Mean 27-30	25.7					Mean 32-164e	2.2

[a] Data for tetramate analogues were consistently obtained from 400 MHz NMR spectroscopy with samples in CDCl₃.

Tetramate carboxamides **27-30** with C-2 pyridyl rings, as described in Chapter 2, were strongly metal binding, with an average post-column H-2 width of 25.7 Hz (Table 5.2). The variations of peak broadening across the series suggested that C-2 pyridyl rings also had different metal binding abilities. Depending on the nature of the coordinated metal ions

and tautomeric behaviour of the metal chelates, tetramate analogues can bind to metal with C-6/C-9 oxygen or C-8/C-9 oxygen.⁴⁷ Direct functionalisation at C-6 and C-9 detailed in Chapter 3 drastically reduced metal binding as tetramate derivatives **32**, **35**, **36-56** and **164e** had an average post-column H-2 width of only 2.2 Hz (Table 5.2). The significant improvement in post-column peak resolution was visually discernable (section 3.4, page 103-104, *vide supra*). This supported the direct involvement of C-6 and C-9 oxygen in metal complexation. The decreased metal chelation ability led to a general loss of antibacterial activity, suggesting that in this series of tetramate analogues, modes of action were metal-binding dependent. These tetramate derivatives could bind to divalent metal ion cofactors such as Mg²⁺, which is most commonly present in many bacterial enzymes including UPPS, RNAP, topoisomerases and DNA gyrase for target interactions.^{148,212,213} It was noted that the enolisable tetramate structure and the resultant acidic proton were important for metal chelation and biological activity. This nevertheless validated the concept of prodrug design through functionalisation at C6/C9 to access non-metal chelating tetramate derivatives, which could exert their biological activities when metabolised *in vivo*. Interestingly in the case of tetramate derivative **41e**, it was non-metal binding and demonstrated good antibacterial activity, with lowest MIC value against MRSA at 1.95 µg/ml, indicating a different mode of action independent from metal chelation (Figure 5.4).

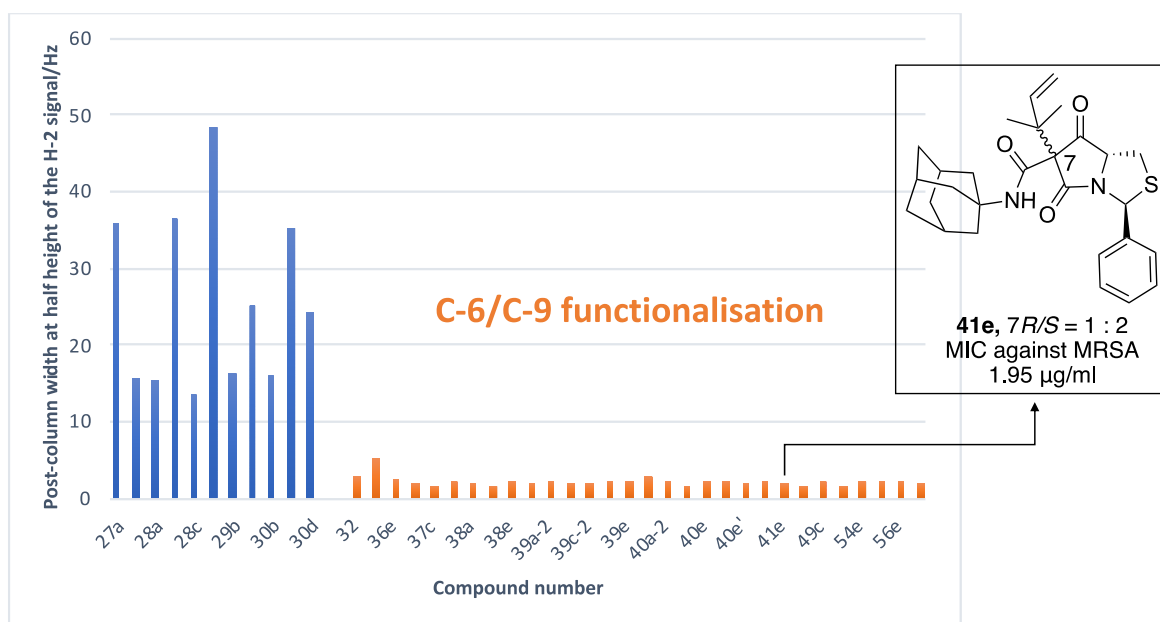


Figure 5.4 Plot of post-column H-2 widths at half height ($W_{1/2}$, Hz) for tetramate analogues **27-30** and derivatives with C-6/C-9 functionalisation (**32, 35, 36-56** and **164e**).

The principle behind chemical modifications of C-5 esters as described in Chapter 4 was to reduce metal chelation through steric influence of the neighbouring C-5 groups. The steric bulkiness of the C-5 esters was indicated by their van der Waals volumes (\AA^3) and correlated with the post-column H-2 widths at half height (Table 5.3 for tetramate carboxamides and Table 5.4 for tetramate ketones). In general, an increase in the bulkiness of C-5 ester was correlated with a decrease in peak broadening, suggesting a reduced metal-binding ability and hence validating the working principle behind chemical modifications detailed in Chapter 4.

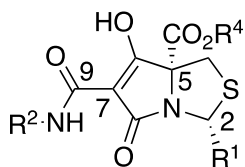
For tetramate carboxamides with C-5 esters (**145-159** and **162**, Table 5.3), it was noted that post-column peak broadening could also be complicated by tautomeric behaviour as each of them appeared to be an approximately equimolar mixture of two tautomers in

CDCl₃. However, with conserved H-2 chemical shifts and tautomeric ratios throughout the series, the decreasing trend of peak broadening with increased bulkiness of C-5 groups was attributed to decreased metal binding. The case of tetramate carboxamide **153e** appeared to be an outlier, with nearly 3-fold larger width at half height than the value predicted by the best fit line, at least suggesting that C-5 allyl ester was not suitable to reduce metal chelation (Table 5.3). Tetramate analogues with heterocycles at C-2, C-5 (**162**) or C-9 pendants were generally more metal-binding as heterocycles could also participate in extended metal chelation (Table 5.3). The trend of metal binding properties in tetramate carboxamides with C-2 pyridyl rings was also less obvious and made more complex by the different metal chelating abilities of heterocyclic rings (Table 5.3). The smallest extent of peak broadening among polar heterocycle-containing tetramate carboxamides was observed with **155a** with C-2 pyridin-2-yl ring and C-5 cyclohexyl ester (Table 5.3).

Similarly, tetramate ketones with C-2 pyridyl rings often exhibited significant post-column broadening of signals to give no distinguishable H-2 peaks for calculation. Among C-2 phenyl-substituted tetramate ketones (Table 5.4), **112e**, **130e** and **123e** with C-5 methyl esters did not appear to fit in the linear downward trend with lower-than-expected peak broadening, but their lack of antibacterial activity against MRSA (**112e** was inactive; **130e** and **123e** had an MIC value of 15.6 µg/ml) suggested that C-5 methyl ester was not the optimal group to achieve reduced metal chelation while preserving good antibacterial activity (Figure 5.3, page 169, *vide supra*). It was noted that the C-9 pendant in tetramate ketones played an important role in reducing metal chelation, giving a drastic decrease in post-column peak broadening in C-9 cyclohexyl tetramate ketones as compared to C-9

ethyl or phenethyl tetramate ketones of the same C-5 ester (Table 5.4). The best combination for reducing metal chelation was in the case of **128e** with C-9 cyclohexyl ketone pendant and C-5 benzyl ester, with a post-column H-2 width at 2.4 Hz, which is almost as sharp as those non-metal binding tetramate analogues with direct functionalisation at C-6/C-9.

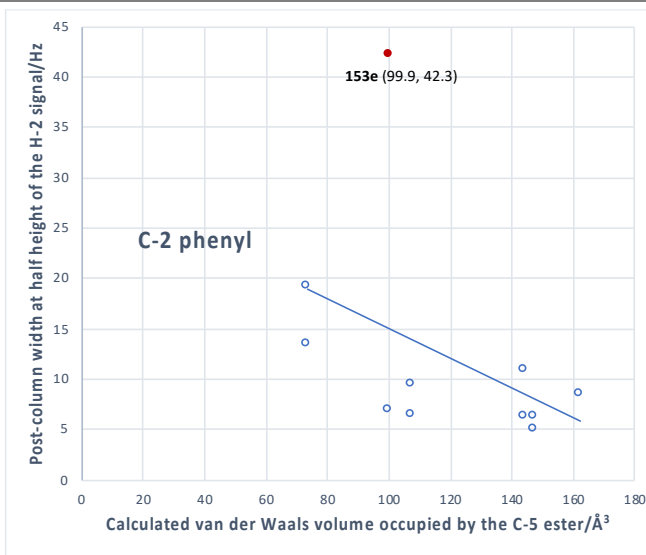
Table 5.3 Post-column widths at half height/Hz for H-2 signals of tetramate amides.



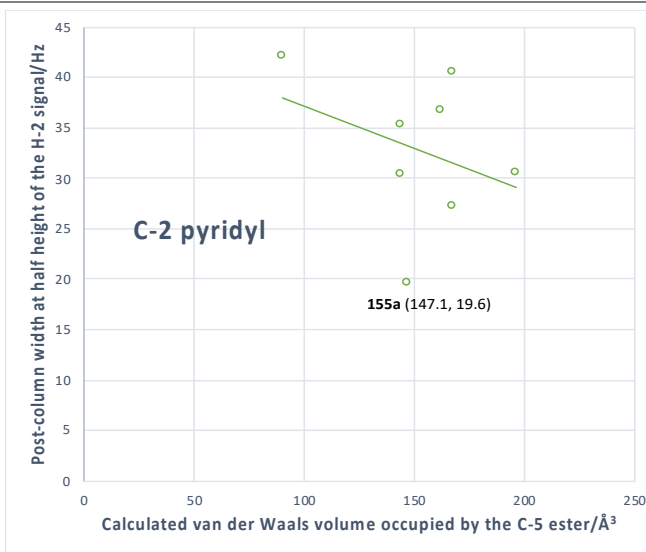
145-159, 162

R ¹ = a = pyridin-2-yl	R ⁴ = methyl, allyl, isopropyl, cyclohexyl, benzyl, 4-nitrobenzyl, 2-nitrobenzyl, 4-bromobenzyl, 4-(furan-2-yl)benzyl
b = 2-bromo-pyridin-4-yl	
c = 5-bromo-pyridin-2-yl	
e = phenyl	
R ² = 145-150 = cyclohexyl	
151-159, 162 = 1-adamantyl	

Cpd No.	Volume occupied by C-5 ester ^a /Å ³	W _{1/2} /Hz
145e	73.5	19.3
146e	99.9	6.9
147e	107.3	9.5
148e	147.1	6.4
149e	144.1	10.9
150e	162.3	8.5
151e	73.5	13.4
153e	99.9	42.3
154e	107.3	6.5
155e	147.1	5.1
156e	144.13	6.34



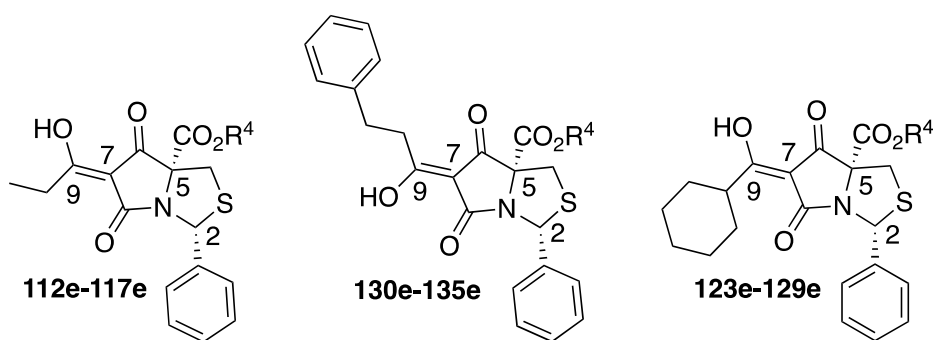
Cpd No.	Volume occupied by C-5 ester ^a /Å ³	W _{1/2} /Hz
149c	144.1	35.3
152b	90.3	42.0
155a	147.1	19.6
156c	144.1	30.4
157a	167.1	40.5
158b	167.1	27.2
159c	162.3	36.7
162	196.0	30.4



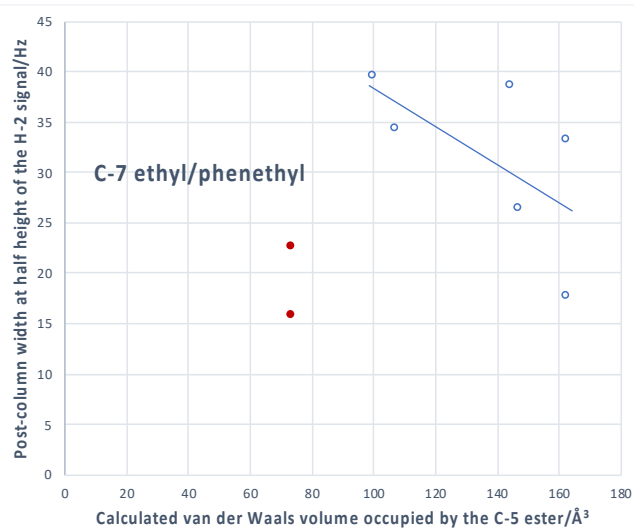
[a] Van der Waals volume occupied by C-5 ester calculated by geometrical analysis in

Marvin (20.3.0), 2020, ChemAxon.

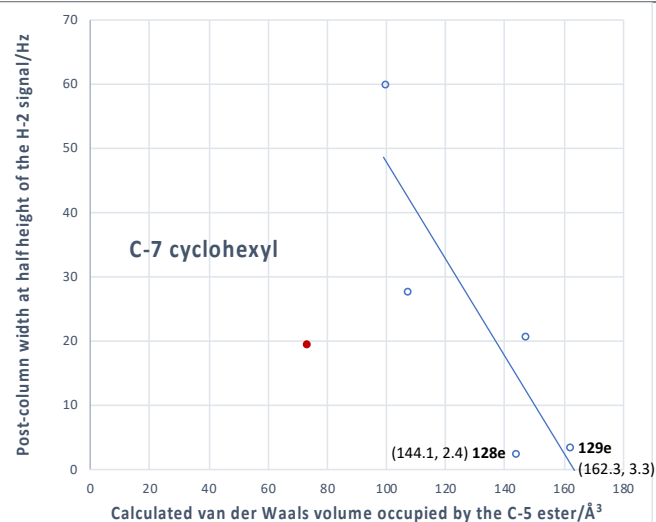
Table 5.4 Post-column widths at half height/Hz for H-2 signals of tetramate ketones.



Cpd No.	Volume occupied by C-5 ester ^a /Å ³	W _{1/2} /Hz
112e	73.5	15.8
113e	99.9	39.6
114e	107.3	34.3
115e	147.1	26.4
116e	144.1	38.5
117e	162.3	33.3
130e	73.5	22.6
135e	162.3	17.6



Cpd No.	Volume occupied by C-5 ester ^a /Å ³	W _{1/2} /Hz
123e	73.5	19.4
125e	99.9	59.8
126e	107.3	27.6
127e	147.1	20.6
128e	144.1	2.4
129e	162.3	3.3



Interestingly, unlike C-6/C-9 modified tetramates, reduced metal chelation ability in the C-5 modified tetramate analogues did not result in a detrimental loss of antibacterial activity (Figure 5.3, page 169, *vide supra*). The C-5 modified tetramate analogues that

showed some antibacterial activity with MIC values against MRSA ≤ 62.5 $\mu\text{g/ml}$ were selected, and their MIC values were plotted against post-column peak broadening ($W_{1/2}/\text{Hz}$ of the H-2 signal) (Figure 5.5). The absence of correlation between activity and peak broadening shown by Figure 5.5 indicated that among tetramate analogues with C-5 esters, antibacterial activity does not depend on metal chelation, with **128e**, **129e**, **148e**, **154e** and **155a** demonstrating direct evidence of reduced metal binding while exhibiting good antibacterial activity with MIC values against MRSA ≤ 7.81 $\mu\text{g/ml}$.

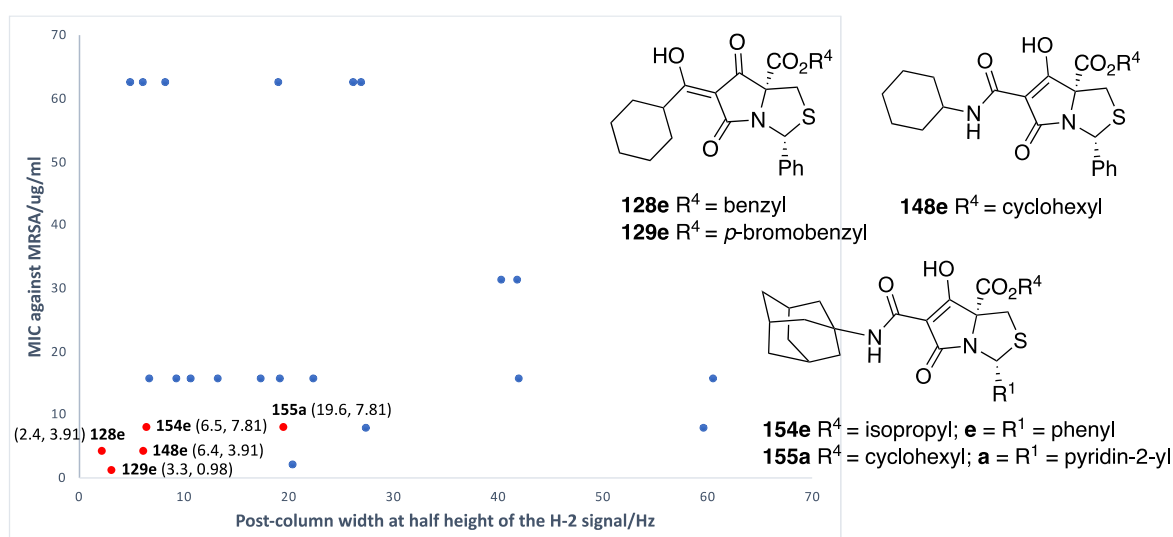
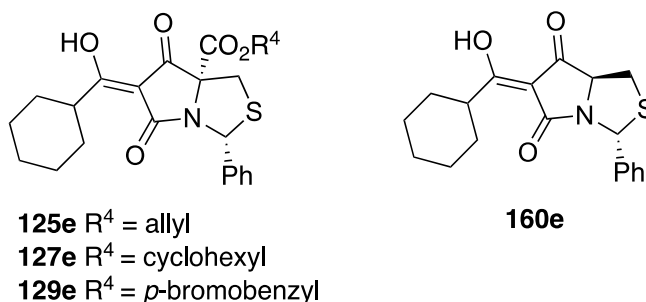


Figure 5.5 Plot of MIC values against MRSA ($\mu\text{g/ml}$) against post-column widths at half height of the H-2 signal (Hz) for C-5 modified tetramate analogues

Initial results from bioassays in presence of metal ions demonstrated that antibacterial activity was maintained for these C-5 modified tetramate ketones (Table 5.5). This was in stark contrast with tetramate carboxamides **28c** and **30a-d**, whose antibacterial activities (MIC against MRSA ranging from 0.24 to 7.81 $\mu\text{g/ml}$) were completely lost in the presence of 0.01% Fe^{3+} solution. Although it was disappointing to observe loss of activity in presence of serum/blood, it was likely to be due to plasma protein binding with the four

compounds possessing relatively high lipophilicity (Table 5.5). As this work is still in progress, it would be interesting to see the results for those C-5 modified tetramate analogues with improved physicochemical properties.

Table 5.5 Initial results of antibacterial activity in presence of metal ions/blood.



Cpd. No	MIC against MRSA ($\mu\text{g/ml}$) in presence of			clogP	PSA	% PSA	
	-	metal ions ^[a]	HSA ^[b]				blood ^[c]
125e	7.81	7.81	-	-	3.56	83.91	14.6
127e	3.91	7.81	-	-	4.41	83.91	12.7
129e	1.95	0.98	-	-	5.39	83.91	12.6
160e	15.6	15.6	-	-	3.14	57.61	12.4

[a]Medium with Fe³⁺, Ca²⁺, Mg²⁺ and Zn²⁺ in the same concentrations as in blood; [b]

human serum albumin (HSA) in the same concentration as in blood; [c] medium with metal ions and HSA in the same concentrations as in blood.

5.4 A brief note on cytotoxicity

Safety in human use is another important selection criterion for good antibacterial drugs.³⁰ Natural product inspired structures are often reported with good biological activities coupled with low toxicity,^{63,214,215} which also motivated the synthesis of tetramate derivatives in this project. As the bicyclic tetramate core contains α,β -unsaturated carbonyl functional moieties, a concern for toxicity was raised with regard to whether it can act as a Michael acceptor for nucleophilic interactions with thiol-

containing proteins, DNA and glutathione, all of which play important roles for cell survival and metabolism.²¹⁶ However, a preliminary evaluation of tetramate ester **25c** and carboxamide **28c** indicated that no reaction with L-cysteine methyl ester was observed over 24 h in methanol-*d*₄ (Figure 5.6), suggesting a low electrophilic reactivity of these tetramate analogues.

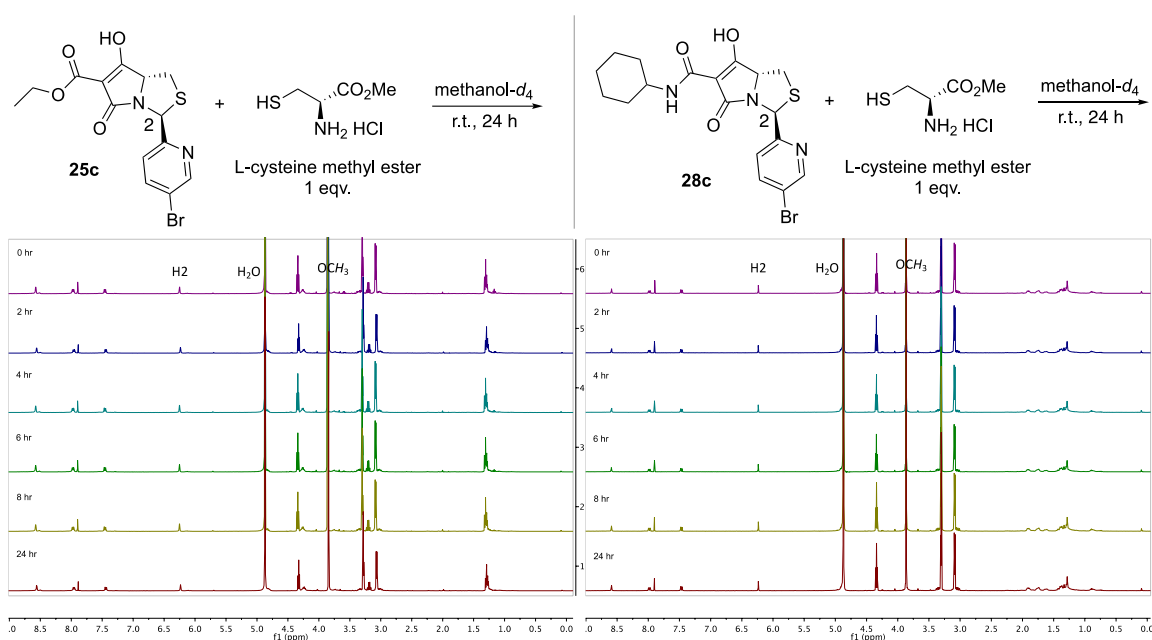


Figure 5.6 NMR monitoring of tetramate analogues **25c** and **28c** with L-cysteine methyl ester in methanol-*d*₄ at room temperature over 24 h.

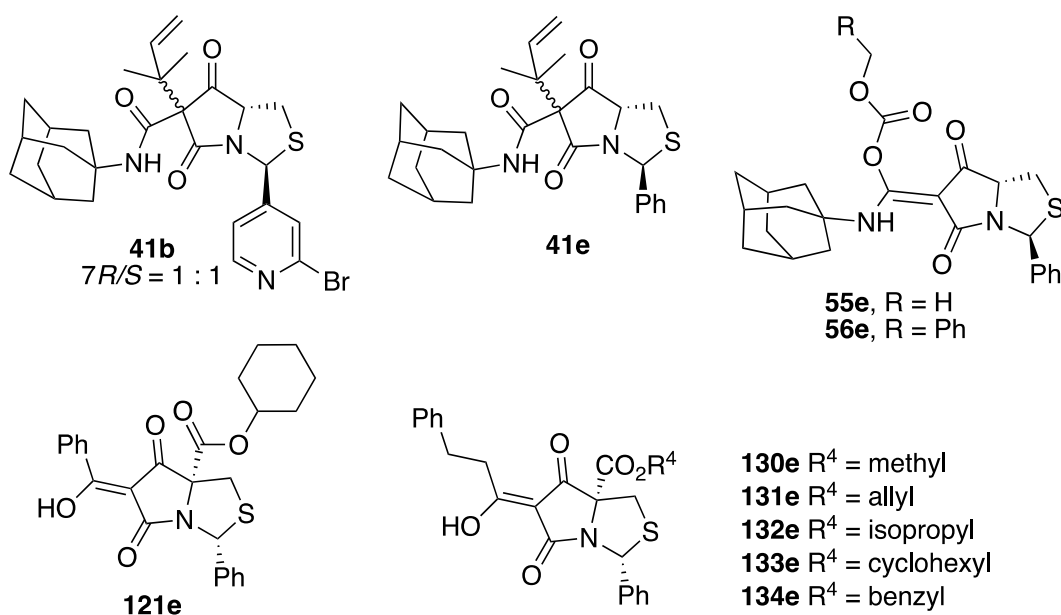
The selectivity of synthesised tetramate analogues to prokaryotic bacteria cells over eukaryotic mammalian cells was analysed using cytotoxicity screens against Hela, HEK-239, CaCo and MDCK cell lines (conducted in collaboration with Oxford Antibiotic Group). As for the choice of cell lines used for cytotoxicity analysis, Hela is a highly durable and proliferative human cervical cancer cell line;²¹⁷ HEK-239 is a human embryonic kidney cell line to model renal toxicity; CaCo is a human colorectal adenocarcinoma cell line as a

model of intestinal epithelium²¹⁸ to test for gastrointestinal toxicity; and MDCK is a dog kidney cell line as a non-human mammalian model.

For interpretation of MIC values, an MIC against MRSA of < 8 µg/ml was considered active.³⁶ An IC₅₀ of 62.5 - 125 µg/ml against selected cell lines in the cytotoxicity screen was considered to be non-toxic, with the weak toxicity more likely to be contributed by DMSO introduced during the testing procedure;^{219,220} an IC₅₀ of 15.6 - 31.3 µg/ml was considered less toxic while a IC₅₀ < 8 µg/ml was toxic. The selectivity index for MRSA was calculated as the ratio of IC₅₀ in respective cell lines against the MIC value (IC₅₀/MIC).

For the tested tetramate analogues, **131e-134e** demonstrated good selectivity towards MRSA, with **131e** and **133e** being more active with a MIC value of 0.98 µg/ml and selectivity of more than 8-fold. While **56e**, **132e** and **134e** were less active, they were also generally less toxic, with selectivity for MRSA as good as 8-fold. The different cytotoxicity towards the tested cell lines also suggested that there could be a therapeutic window for these tetramate analogues with specific tissue toxicities at high dose. It was noted that reduction in toxicity could be achieved by increasing compound complexity with introduction of sp³ hybridised carbon and reduction of aromatic rings to improve specificity of target interaction.^{221,222} In this regard, the chiral bicyclic tetramate template was an attractive scaffold to start with and among the tetramate analogues tested for cytotoxicity, tetramate ketones **131e** and **133e** were promising leads for further optimisation.

Table 5.6 Cytotoxicity screen of selected cysteine-derived tetramate analogues.



	MIC ($\mu\text{g/ml}$) against MRSA	IC ₅₀ ($\mu\text{g/ml}$) against				MRSA Selectivity
		Hela	HEK- 239	CaCo	MDCK	
41b	15.6	7.81	7.81	31.3	31.3	0.5 - 2.0
41e, 7R/S = 1:2	1.95	3.91	3.91	7.81	7.81	2.0 - 4.0
41e, 7R/S = 1:1.5	3.91	7.81	7.81	15.6	15.6	2.0 - 4.0
55e	31.3	31.3	62.5	62.5	31.3	1.0 - 2.0
56e	15.6	15.6	31.3	125	62.5	1.0 - 8.0
121e	31.3	31.3	31.3	31.3	62.5	1.0 - 2.0
130e	15.6	31.3	31.3	31.3	62.5	2.0 - 4.0
131e	0.98	7.81	15.6	7.81	7.81	8.0 - 16.0
132e	3.91	15.6	31.3	15.6	31.3	4.0 - 8.0
133e	0.98	7.81	15.6	7.81	15.6	8.0 - 16.0
134e	3.91	31.3	31.3	31.3	31.3	8.0

5.5 Summary and conclusion

This chapter described the evaluation of the synthesised tetramate derivatives as antibacterial drugs. Tetramate analogues with C-9 hydrophobic amide/ketone pendants exhibited promising antibacterial potencies against Gram-positive MRSA with MIC values $< 8 \mu\text{g/ml}$. Analysis of their physicochemical properties delineated a chemical space occupied by the active tetramate analogues, with MSA of 482 - 746 Å², MW of 400 - 577 Da, clogP of 0.7 - 7.0, clogD_{7.4} of -1.7 - 5.0, PSA of 66 - 109 Å² and relative PSA of 9 - 17%. The structure-activity relationships indicated that clogP and clogD_{7.4} of the active tetramate analogues change approximately in proportion to MW while relative PSA changes in the inverse direction to MW and there is a narrow activity window for PSA variations. The active tetramate derivatives are generally Lipinski's rule compliant, which predict for good oral availability. The set of physicochemical properties fit to the chemical space of antibacterial drugs that possess selective Gram-positive activity. The observation that Gram-positive and Gram-negative activities require different sets of physicochemical properties might be related to bacterial cell permeability. For structural modifications on the bicyclic tetramate template, incorporation of polar heterocycles at C-2 and/or introduction of C-5 esters resulted in an improvement of hydrophilicity to reduce plasma protein binding while preserving good antibacterial activity. Modifications at C-6/C-9 of tetramate analogues led to a complete loss of metal chelating ability, which was correlated with a loss of antibacterial activity. A C-7 alkylated tetramate derivative with no metaling binding ability was found to be active with an MIC against MRSA $\leq 1.95 \mu\text{g/ml}$, suggesting a mechanism of action independent from metal chelation. Introduction of bulkier C-5 esters generally resulted in reduced metal chelation abilities, with direct evidence from smaller extent of post-column peak broadening of ¹H NMR spectra.

Antibacterial activities of C-5 modified tetramate analogues were also independent from metal chelation, with examples of tetramate ketones and carboxamides demonstrating both reduced metal binding and good antibacterial activity with MIC values against MRSA $\leq 7.81 \mu\text{g/ml}$. The antibacterial activity of these tetramate analogues was maintained when tested in medium with metal ions of the same concentrations as in blood. A subset of tetramate analogues were tested for their cytotoxicity in mammalian cells with examples of tetramate analogues exhibiting good selectivity towards prokaryotic bacterial cells. Overall, this project demonstrated the suitability of the natural product inspired bicyclic tetramate template as a promising structural motif for the development novel antibacterial drugs, with good anti-MRSA potencies and appropriate physiochemical properties to be drug-like, coupled with a potential for multi-targeting mechanisms and low eukaryotic cytotoxicity.

Chapter 6 Experimental

6.1 General techniques

All reagents were obtained either from Sigma Aldrich, Alfa Aesar or Fluorochem and used without further purification. All reactions were carried out in oven-dried reaction flasks under inert (N₂) atmosphere unless not using dry solvents. Reaction times were recorded in minutes (min), hours (h) or days (d). Reactions left overnight (o.n.) lasted for 16 - 20 h. 'Petroleum ether' refers to that fraction of light petroleum ether boiling at 40-60 °C and was used as received. Temperatures below room temperature were obtained using cold baths: 0 °C (ice/water), -15 °C (ice/NaCl salt) and -78 °C (dry ice/acetone). Temperatures above room temperature were obtained with heating with oil bath. Solvents were evaporated at 40 °C unless otherwise stated under reduced pressure on a Buchi RE 111 Rotavapour attached to a Vacuubrand CVC2 pump and pressure control system. Concentrations (c) in the general procedures referred to the limiting reagent and were given in mmol/mL.

Thin layer chromatography (TLC) was performed using Merck aluminium foil backed sheets precoated with 0.2 mm Kieselgel 60 F₂₅₄. Product spots were visualized by UV fluorescence (max 254 nm, for conjugated systems), staining with a KMnO₄ solution and heating (for unsaturated systems) or staining with ninhydrin solution and heating (for primary and secondary amines). Retention factors (*R_f*) were quoted to the nearest 0.01. Column chromatography was carried out using Sigma Aldrich silica gel 60, 0.040-0.063 (230-400 mesh particle size). The eluents used were determined based on the *R_f* values.

Melting points were recorded using a Stuart Scientific SMP1 melting point instrument in open capillaries and were uncorrected.

Optical rotations were recorded on a Perkin-Elmer 241 polarimeter at the stated temperature (25 °C) using the D line of sodium (wavelength at 589 nm) and a path length of 1 dm. Specific rotations $[\alpha]_D^{25}$ were calculated and reported in $10^{-1} \text{ } ^\circ\text{C cm}^2 \text{ g}^{-1}$ with concentration c given in g/100 ml.

Infrared (IR) spectra were recorded on a Bruker Tensor 27 ATR FT-IR spectrometer with thin film (oil sample prepared in 10 mg/1ml CHCl_3) or powder (solid). Absorption maxima (ν_{max}) were reported in wavenumbers (cm^{-1}) and only selected peaks were reported.

^1H NMR spectra were recorded on AVF (400 MHz) or AVC (500 MHz) spectrometers. ^{13}C NMR spectra were recorded on Bruker AVF spectrometer at 101 MHz or on AVC spectrometer at 126 MHz with proton decoupling and cryogenic detection probe. Chemical shifts (δ) were reported in parts per million (ppm) and were referenced to the residual solvent peak in ^1H NMR or deuterated solvent peak in ^{13}C NMR. The abbreviations used to describe multiplicity were as follows: s (singlet), br. s (broad singlet), d (doublet), dd (double doublet), t (triplet), td (triple doublet), dt (doublet triplet), q (quartet), sept (septet), m (multiplet). Coupling constants (J) were given in Hertz (Hz). 2D-NOESY and 1D-NOE experiments were performed using Bruker AVB400 or AVC (500 MHz) spectrometer. HMBC experiments were recorded on Bruker AVC spectrometer.

Low resolution mass spectra (m/z) were recorded on a Fisons Platform spectrometer using electrospray ionisation (ESI). High resolution mass spectra (HRMS) were recorded on a Bruker μ TOF (ESI or APCI) spectrometer by the internal service at the Department of Chemistry, University of Oxford. The m/z values of major peaks were reported in Daltons and their intensities given as percentages of the base peaks. When halogens ($X = \text{Cl}$ or Br) are present, MS peaks for isotopomers were included, with the relative abundance corresponded to the isotopic ratios of $^{35}\text{Cl} : ^{37}\text{Cl} = 3 : 1$ and $^{79}\text{Br} : ^{81}\text{Br} = 1 : 1$.

Screening of compounds were performed by Oxford Antibiotic Group, Austria. For MIC determination by broth dilution assay, the samples were tested in a primary 96 well plates screening assay. The compounds were diluted in Mueller Hinton Broth (MHB) for bacterial screening to a stock solution of 1000 $\mu\text{g}/\text{mL}$, serial diluted and overlaid with a microbe solution in a concentration of 10^4 CFU/ml. The plates were incubated for 24 h at 35 °C, after which MIC values were read from the plates.

For cytotoxicity testing, the synthesized compounds were tested against four different cell lines: HeLa, HEK 293, MDCK and CaCo. The cells were seeded in a 96 well plate and incubated until a confluence of 80% was achieved (under physiological conditions - 37°C, 5% CO_2 and 95% humidity). The samples were tested by serial dilution in triplicates with starting concentration of 250 $\mu\text{g}/\text{mL}$. After 24 and 48 hours the survival of cells was evaluated by microscope and measured with Alamar blue. IC_{50} values were obtained from the calibration curves.

6.2 Experimental for Chapter 2

6.2.1 General procedure (A) for synthesis of thiazolidines 23a-d

Et₃N (1.2 eqv.) and the respective aldehyde (1.0 eqv., 2 g/10 mmol) were added to L-cysteine methyl ester hydrochloride (1.2 eqv.) in petroleum ether (*c* = 0.3). The mixture was heated under reflux at 110 °C with continuous removal of water using Dean-Stark apparatus for 18 h. The reaction mixture was then filtered and washed with diethyl ether and the combined filtrates were concentrated *in vacuo* and residue was purified by flash column chromatography to give thiazolidines **23a-d**.

6.2.2 General procedure (B) for synthesis of *N*-acylthiazolidines 24a-d

Mono-ethyl malonate (1.2 eqv.) was added dropwise to a stirred solution of thiazolidine (1.0 eqv., 9 mmol), *N,N'*-dicyclohexylcarbodiimide (DCC, 1.2 eqv.) and 4-dimethylaminopyridine (DMAP, 0.1 eqv.) in DCM (*c* = 0.2) at 0 °C. The mixture was stirred at 0 °C for 15 min and then at room temperature for 18 h. The reaction mixture was filtered and washed with DCM and the combined filtrates were concentrated *in vacuo* and residue was purified by flash column chromatography to give *N*-acylated thiazolidines **24a-d**.

6.2.3 General procedure (C) for synthesis of tetramate esters 25a-d

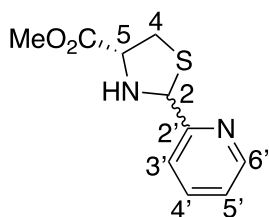
Potassium *tert*-butoxide (KO^tBu, 1.2 eqv.) was added to a stirred solution of *N*-acylated thiazolidine (1.0 eqv., 8 mmol) in dry THF (*c* = 0.1) and the mixture was heated under reflux for 4 h. It was then cooled to room temperature, concentrated *in vacuo* and partitioned between diethyl ether and water. The aqueous layer was carefully acidified with 2M hydrochloric acid (HCl) to pH = 3 and extracted with ethyl acetate (EtOAc). The

combined organic layers were washed with brine, dried with MgSO_4 , filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography with 1% Et_3N in the eluent to give the desired product. The product was dissolved in DCM and washed with 5% citric acid. The combined organic fractions were dried with MgSO_4 , filtered and concentrated *in vacuo* to yield the desired tetramate esters **25a-d**.

6.2.4 General procedure (D) for synthesis of tetramate carboxamides **27-30(a-d)**

Amine (1.2 eqv.) was added to a stirred solution of tetramate ester (1.0 eqv., 200 mg) in THF/toluene (1 : 4, $c = 0.05$) and the mixture was heated at reflux for 18 h. It was then cooled to room temperature, concentrated *in vacuo* and residue was purified by flash column chromatography with 1% Et_3N in the eluent to give the desired product. The product was dissolved in DCM and washed with 5% citric acid. The combined organic fractions were dried with MgSO_4 , filtered and concentrated *in vacuo* to yield the desired tetramate carboxamides **27-30(a-d)**.

(5*R*)-5-Methoxycarbonyl-2-(pyridin-2-yl)-1,3-thiazolidine, **23a**

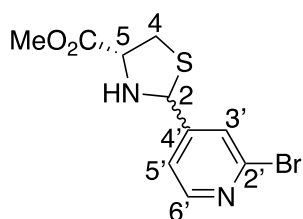


Yield (1.95 g, 75%); orange oil; inseparable 1.2 : 1 *cis* and *trans* diastereomers; $R_f = 0.44$ (petrol : EtOAc; 1 : 4); $\nu_{\text{max}}/\text{cm}^{-1}$ 1740 (s, C=O), 3291 (br, N-H); $^1\text{H NMR}$ (CDCl_3 , 500 MHz): major isomer (*cis*):

δ (NH not observed), 8.62 (d, $J = 4.8$ Hz, 1H, H6'), 7.67 (dd, $J = 7.8, 1.8$ Hz, 1H, H4'), 7.29 (d, $J = 7.8$ Hz, 1H, H3'), 7.20 – 7.27 (m, 1H, H5'), 5.65 (s, 1H, H2), 4.03 (dd, $J = 9.7, 6.7$ Hz, 1H, H5), 3.82 (s, 3H, CO_2CH_3), 3.44 (dd, $J = 10.1, 6.7$ Hz, 1H, H4_B), 3.09 (app t, 1H, $J = 9.9$ Hz, H4_A); minor isomer (*trans*): δ (NH not observed), 8.57 (d, $J = 4.8$ Hz, 1H, H6'), 7.63 – 7.66 (m, 1H, H4'), 7.33 (d, $J = 7.8$ Hz, 1H, H3'), 7.16 – 7.23 (m, 1H, H5'), 5.84 (s, 1H, H2), 4.55

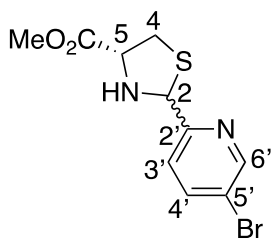
(dd, $J = 6.6, 4.4$ Hz, 1H, H5), 3.80 (s, 3H, CO₂CH₃), 3.32 – 3.40 (m, 2H, H4_A and H4_B); ¹³C NMR (CDCl₃, 101 MHz): major isomer (*cis*): δ 170.8 (CO₂CH₃), 156.4 (C2'), 149.4 (C6'), 136.5 (C4'), 123.0 (C3'), 121.7 (C5'), 71.2 (C5), 65.8 (C2), 52.2 (CO₂CH₃), 39.0 (C4); minor isomer (*trans*): δ 171.8 (CO₂CH₃), 158.3 (C2'), 149.2 (C6'), 136.5 (C4'), 122.6 (C5'), 121.2 (C3'), 70.7 (C5), 65.3 (C2), 52.2 (CO₂CH₃), 38.4 (C4); m/z (ESI⁺) 225 ([M+H]⁺, 100%); m/z (ESI⁺) 225 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z : [M+H]⁺ Calcd for C₁₀H₁₃O₂N₂S 225.0692; Found 225.0690.

(5*R*)-2-(2-Bromopyridin-4-yl)-5-methoxycarbonyl-1,3-thiazolidine, 23b



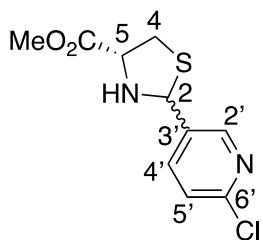
Yield (3.04 g, 86%); yellow oil; inseparable 0.6 : 1 *cis* and *trans* diastereomers; $R_f = 0.15$ (petrol : EtOAc; 4 : 1); $\nu_{\max}/\text{cm}^{-1}$ (neat) 1736 (s, C=O), 3315 (br, N-H); ¹H NMR (CDCl₃, 400 MHz): major isomer (*trans*): δ (NH not observed), 8.29 (d, $J = 5.2$ Hz, 1H, H6'), 7.61 (s, 1H, H3'), 7.32 (d, $J = 5.2$ Hz, 1H, H5'), 5.75 (s, 1H, H2), 3.99 (q, $J = 8.1, 7.0$ Hz, 1H, H5), 3.81 (s, 3H, CO₂CH₃), 3.35 (dd, $J = 10.6, 7.0$ Hz, 1H, H4_B), 3.04 – 3.16 (m, 1H, H4_A); minor isomer (*cis*): δ (NH not observed), 8.37 (d, $J = 5.2$ Hz, 1H, H6'), 7.65 (s, 1H, H3'), 7.38 (d, $J = 5.2$ Hz, 1H, H5'), 5.47 (s, 1H, H2), 3.99 (q, $J = 8.1, 7.0$ Hz, 1H, H5), 3.81 (s, 3H, CO₂CH₃), 3.46 (dd, $J = 10.2, 7.0$ Hz, 1H, H4_B), 3.04 – 3.16 (m, 1H, H4_A); ¹³C NMR (CDCl₃, 101 MHz): major isomer (*trans*): δ 171.6 (CO₂CH₃), 154.7 (C6'), 150.0 (C4'), 142.3 (C2'), 125.7 (C3'), 121.0 (C5'), 68.0 (C5), 64.0 (C2), 52.7 (CO₂CH₃), 38.1 (C4); minor isomer (*cis*): δ 171.0 (CO₂CH₃), 150.6 (C6'), 150.3 (C4'), 142.4 (C2'), 126.5 (C3'), 121.5 (C5'), 69.6 (C5), 65.4 (C2), 52.7 (CO₂CH₃), 38.7 (C4); m/z (ESI⁺) 303 and 305 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z : [M+H]⁺ Calcd for C₁₀H₁₂O₂N₂BrS 302.9797 and 304.9777; Found 302.9795 and 304.9772.

(5R)-2-(5-Bromopyridin-2-yl)-5-methoxycarbonyl-1,3-thiazolidine, 23c



Yield (2.83 g, 80%); yellow oil; inseparable 1 : 1 *cis* and *trans* diastereomers; $R_f = 0.31$ (petrol : EtOAc; 3 : 1); $\nu_{\max}/\text{cm}^{-1}$ 1736 (s, C=O), 3301 (br, N-H); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): *trans* isomer: δ (NH not observed), 8.60 (d, $J = 2.3$ Hz, 1H, H6'), 7.77 (td, $J = 8.1, 2.3$ Hz, 1H, H4'), 7.20 (d, $J = 8.1$ Hz, 1H, H3'), 5.80 (s, 1H, H2), 4.47 (dd, $J = 6.7, 4.7$ Hz, 1H, H5), 3.79 (s, 3H, CO_2CH_3), 3.42 (dd, 1H, $J = 10.6, 6.7$ Hz, H4_B), 3.28 (dd, 1H, $J = 10.6, 4.7$ Hz, H4_A); *cis* isomer: δ (NH not observed), 8.66 (d, $J = 2.3$ Hz, 1H, H6'), 7.77 (td, $J = 8.3, 2.3$ Hz, 1H, H4'), 7.25 (d, $J = 8.3$ Hz, 1H, H3'), 5.60 (s, 1H, H2), 4.01 (dd, $J = 9.6, 6.8$ Hz, 1H, H5), 3.81 (s, 3H, CO_2CH_3), 3.35 (dd, $J = 10.6, 6.8$ Hz, 1H, H4_B), 3.02 – 3.09 (m, 1H, H4_A); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): *cis* and *trans* isomers: δ 172.0, 171.1 (CO_2CH_3), 157.8, 155.5 (C2'), 151.0, 150.7 (C6'), 139.4 (C4'), 123.3, 122.6 (C3'), 120.2, 119.7 (C5'), 70.9, 70.4 (C5), 66.3, 65.6 (C2), 52.7 (CO_2CH_3), 39.5, 38.8 (C4); m/z (ESI⁺) 303 and 305 ($[\text{M}+\text{H}]^+$, 100%); HRMS (ESI⁺) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{10}\text{H}_{12}\text{O}_2\text{N}_2\text{BrS}$ 302.9797 and 304.9777; Found 302.9800 and 304.9777.

(5R)-2-(6-Chloropyridin-3-yl)-5-methoxycarbonyl-1,3-thiazolidine, 23d

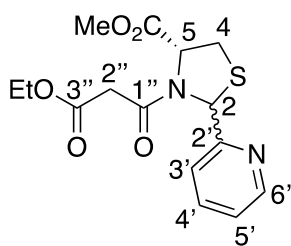


Yield (2.49 g, 83%); yellow oil; inseparable 0.8 : 1 *cis* and *trans* diastereomers; $R_f = 0.48$ (petrol : EtOAc; 3 : 2); $\nu_{\max}/\text{cm}^{-1}$ 1736 (s, C=O), 3304 (br, N-H); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): major isomer (*trans*): δ (NH not observed), 8.43 (s, 1H, H2'), 7.73 (d, $J = 8.3$ Hz, 1H, H4'), 7.23 (d, $J = 8.3$ Hz, 1H, H5'), 5.77 (s, 1H, H2), 4.02 (app t, $J = 6.7$ Hz, 1H, H5), 3.75 (s, 3H, CO_2CH_3), 3.32 (dd, $J = 11.4, 7.0$ Hz, 1H, H4_B), 3.03 – 3.15 (m, 1H, H4_A); minor isomer (*cis*): δ (NH not observed), 8.45 (s, 1H, H2'), 7.82 (d, $J = 8.3$ Hz, 1H, H4'), 7.30 (d, $J = 8.3$ Hz, 1H, H5'), 5.48 (s, 1H, H2), 3.96 (app t, $J = 8.1$ Hz, 1H, H5), 3.75 (s, 3H, CO_2CH_3), 3.43 (dd, $J =$

10.1, 7.2 Hz, 1H, H_{4B}), 3.03 – 3.15 (m, 1H, H_{4A}); ¹³C NMR (CDCl₃, 101 MHz): major isomer (*trans*): δ 171.8 (CO₂CH₃), 150.6 (C2'), 148.5 (C6'), 137.8 (C4'), 136.6 (C3'), 123.8 (C5'), 67.3 (C5), 64.0 (C2), 52.8 (CO₂CH₃), 38.0 (C4); minor isomer (*cis*): δ 171.3 (CO₂CH₃), 151.6 (C2'), 149.0 (C6'), 138.1 (C4'), 133.2 (C3'), 124.3 (C5'), 68.9 (C5), 65.5 (C2), 52.8 (CO₂CH₃), 39.1 (C4); *m/z* (ESI⁺) 259 ([M+H]⁺, 100%) and 261 ([M+H]⁺, 33%); *m/z* (ESI⁺) 259 ([M+H]⁺, 100%) and 261 ([M+H]⁺, 33%); HRMS (ESI⁺) *m/z*: [M+H]⁺ Calcd for C₁₀H₁₂O₂N₂ClS 259.0303 and 261.0273; Found 259.0303 and 261.0271.

(5*R*)-1-(3-Ethoxy-3-oxopropanoyl)-5-methoxycarbonyl-2-(pyridin-2-yl)-1,3-thiazolidine,

24a



Yield (2.41 g, 84%); yellow oil; separable 1.2 : 1 *cis* and *trans* diastereomers; major isomer (*cis*, a 1 : 0.2 mixture of rotamers in CDCl₃): *R_f* = 0.36 (petrol : EtOAc; 1 : 1); [α]_D²⁵ = + 67.7 (*c* = 0.94, CHCl₃); *v*_{max}/cm⁻¹ 1664 (s, C=O), 1740 (s, C=O); ¹H NMR (CDCl₃,

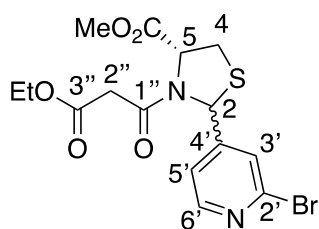
500 MHz): δ 8.55 (d, *J* = 4.8 Hz, 1H, H_{6'} major rotamer), 8.47 (d, *J* = 4.4 Hz, H_{6'} minor rotamer), 8.08 (app d, *J* = 8.0 Hz, 1H, H_{4'} major rotamer), 7.71 – 7.79 (m, 1H, H_{3'} major rotamer), 7.59 – 7.63 (m, H_{3'} minor rotamer), 7.23 (dd, *J* = 8.0, 4.8 Hz, 1H, H_{5'} major rotamer), 7.10 – 7.14 (m, H_{5'} minor rotamer), 6.45 (s, H₂ minor rotamer), 6.18 (s, 1H, H₂ major rotamer), 5.07 (dd, *J* = 6.7, 3.7 Hz, H₅ minor rotamer), 5.01 (dd, *J* = 8.3, 6.6 Hz, 1H, H₅ major rotamer), 4.18 (q, *J* = 7.2 Hz, OCH₂CH₃ minor rotamer), 4.11 (q, *J* = 7.1 Hz, 2H, OCH₂CH₃ major rotamer), 3.80 (s, 3H, CO₂CH₃ major rotamer), 3.78 (s, CO₂CH₃ minor rotamer), 3.37 (d, *J* = 15.6 Hz, 1H, H_{2''_B} major rotamer), 3.30 (dd, *J* = 12.0, 6.6 Hz, 1H, H_{4_B} major rotamer), 3.22 (dd, *J* = 12.0, 8.3 Hz, 1H, H_{4_A} major rotamer), 3.13 (d, *J* = 15.6 Hz, 1H, H_{2''_A} major rotamer), 1.26 (t, *J* = 7.2 Hz, OCH₂CH₃ minor rotamer), 1.21 (t, *J* = 7.1 Hz, 3H,

OCH₂CH₃ major rotamer); ¹³C NMR (CDCl₃, 101 MHz): δ 170.5 and 170.1 (CO₂CH₃), 167.0 and 166.4 (C3''), 165.6 and 165.1 (C1''), 159.3 and 158.5 (C2'), 149.7 and 149.1 (C6'), 137.7 and 136.8 (C4'), 123.3 and 122.7 (C3'), 120.7 and 120.4 (C5'), 67.7 and 67.6 (C5), 64.8 and 63.9 (C2), 61.8 and 61.7 (OCH₂CH₃), 53.2 and 52.8 (CO₂CH₃), 42.8 and 42.1 (C2''), 31.8 (C4), 14.1 and 14.1 (OCH₂CH₃); *m/z* (ESI⁺) 339 ([M+H]⁺, 100%); HRMS (ESI⁺) *m/z*: [M+H]⁺ Calcd for C₁₅H₁₉O₅N₂S 339.1009; Found 339.1008.

Minor isomer (*trans*, a 1 : 0.7 mixture of rotamers in CDCl₃): *R_f* = 0.27 (petrol : EtOAc; 1 : 1); [α]_D²⁵ = -117.3 (*c* = 0.99, CHCl₃); *v*_{max}/cm⁻¹ 1661 (s, C=O), 1739 (s, C=O); ¹H NMR (CDCl₃, 400 MHz): δ 8.57 (d, *J* = 4.1 Hz, 1H, H6' major rotamer), 8.51 (d, *J* = 4.6 Hz, 1H, H6' minor rotamer), 7.72 (td, *J* = 7.7, 1.8 Hz, 1H, H4' major rotamer), 7.64 (app td, *J* = 7.7, 1.4 Hz, 1H, H4' minor rotamer), 7.29 (d, *J* = 7.7 Hz, 1H, H3' major rotamer), 7.23 (d, *J* = 7.7 Hz, 1H, H3' minor rotamer), 7.22 – 7.26 (m, 1H, obscured, H5' major rotamer), 7.16 (dd, *J* = 7.7, 4.6 Hz, 1H, H5' minor rotamer), 6.25 (s, 1H, H2 minor rotamer), 6.16 (s, 1H, H2 major rotamer), 5.32 (app d, *J* = 7.1 Hz, 1H, H5 major rotamer), 5.18 (app d, *J* = 6.1 Hz, 1H, H5 minor rotamer), 4.15 – 4.22 (m, 2H, OCH₂CH₃ minor rotamer), 4.09 (q, *J* = 7.2 Hz, 2H, OCH₂CH₃ major rotamer), 3.86 (s, 3H, CO₂CH₃ minor rotamer), 3.80 (s, 3H, CO₂CH₃ major rotamer), 3.83 – 3.89 (dd, 1H, obscured, H4_B minor rotamer), 3.60 (dd, *J* = 12.3, 7.2 Hz, 1H, H4_B major rotamer), 3.39 – 3.45 (m, 3H, H4_A, H2''_A, H2''_B minor rotamer), 3.32 (d, *J* = 15.2 Hz, 1H, H2''_B major rotamer), 3.20 (app d, *J* = 12.4 Hz, 1H, H4_A major rotamer), 3.07 (d, *J* = 15.2 Hz, 1H, H2''_A major rotamer), 1.26 (t, *J* = 7.2 Hz, 3H, OCH₂CH₃ minor rotamer), 1.22 (t, *J* = 7.2 Hz, 3H, OCH₂CH₃ major rotamer); ¹³C NMR (CDCl₃, 101 MHz): δ 170.4 and 169.8 (CO₂CH₃), 167.1 and 166.4 (C3''), 165.1 and 164.7 (C1''), 160.4 and 160.2 (C2'), 150.1 and 149.6 (C6'), 137.5 and 136.8 (C4'), 123.3 and 122.7 (C3'), 119.8 and 119.5 (C5'), 66.7 and

66.2 (C5), 64.2 and 64.0 (C2), 61.8 and 61.6 (OCH₂CH₃), 53.5 and 52.9 (CO₂CH₃), 43.3 and 42.5 (C2''), 31.5 (C4), 14.1 and 14.1 (OCH₂CH₃); *m/z* (ESI⁺) 339 ([M+H]⁺, 100%); HRMS (ESI⁺) *m/z*: [M+Na]⁺ Calcd for C₁₅H₁₈O₅N₂NaS 361.0829; Found 361.0826.

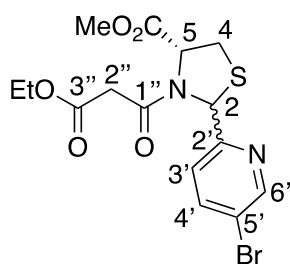
(5*R*)-2-(2-Bromopyridin-4-yl)-1-(3-ethoxy-3-oxopropanoyl)-5-methoxycarbonyl-1,3-thiazolidine, 24b



Yield (3.80 g, 92%); yellow oil; inseparable 1.6 : 1 *cis* and *trans* diastereomers; *R_f* = 0.33 (petrol : EtOAc; 1 : 1); *v*_{max}/cm⁻¹ 1661 (s, C=O), 1738 (s, C=O); ¹H NMR (CDCl₃, 400 MHz): *cis* isomer as a 1 : 0.3 mixture of rotamers, *trans* isomer 1 : 0.8 as a mixture of rotamers in CDCl₃: δ 8.39 (d, *J* = 6.5 Hz, 1H, H6' *trans* isomer), 8.30 (d, *J* = 6.7 Hz, 1H, H6' *cis* isomer), 7.39 (s, 1H, H3' *cis* isomer), 7.34 (s, 1H, H3' *trans* isomer), 7.18 (d, *J* = 6.7 Hz, 1H, H5' *cis* isomer), 7.12 (d, *J* = 6.5 Hz, 1H, H5' *trans* isomer), 6.22 (s, H2 *cis* isomer minor rotamer), 6.14 (s, 1H, H2 *cis* isomer major rotamer), 6.12 (s, 1H, H2 *trans* isomer major rotamer), 6.12 (s, H2 *trans* isomer minor rotamer), 5.20 (app t, *J* = 3.4 Hz, 1H, H5 *cis* isomer major rotamer), 4.19 – 4.28 (m, 2H, OCH₂CH₃ *cis* isomer), 4.08 – 4.19 (m, 2H, OCH₂CH₃ *trans* isomer), 3.86 (s, CO₂CH₃ *cis* isomer minor rotamer), 3.76 (s, CO₂CH₃ *trans* isomer minor rotamer), 3.74 (s, 3H, CO₂CH₃ *cis* isomer major rotamer), 3.72 (s, 3H, CO₂CH₃ *trans* isomer major rotamer), 3.72 – 3.79 (m, 2H, obscured, H4_B), 3.44 – 3.50 (m, 2H, H4_A), 3.12 – 3.39 (m, 4H, H2''_A, H2''_B), 1.31 (t, *J* = 7.0 Hz, 3H, OCH₂CH₃, *trans* isomer major rotamer), 1.22 – 1.28 (m, 3H, OCH₂CH₃, *cis* isomer major rotamer); ¹³C NMR (CDCl₃, 101 MHz): δ 169.9, 169.8, 169.7, 168.9 (CO₂CH₃ major, minor and rotamers), 167.2, 167.1, 166.7, 166.3 (C3'' major, minor and rotamers), 165.9, 165.4, 165.1, 165.0 (C1'' major, minor and rotamers), 153.9, 153.6, 152.4, 151.2 (C6' major, minor and rotamers), 150.9, 150.6, 150.2, 150.0 (C4' major,

minor and rotamers), 143.0, 142.7, 142.3, 142.1 (C2' major, minor and rotamers), 125.9, 125.4, 123.9 (C3' major, minor and rotamers), 120.9, 120.3, 119.3, 119.1 (C5' major, minor and rotamers), 67.8, 67.1, 65.2, 64.7 (C5 major, minor and rotamers), 64.1, 63.9, 63.8, 63.6 (C2 major, minor and rotamers), 62.0, 61.9, 61.7, 61.4 (OCH₂CH₃ major, minor and rotamers), 53.6, 53.4, 52.9, 49.0 (CO₂CH₃ major, minor and rotamers), 43.0, 42.7, 42.3, 42.0 (C2'' major, minor and rotamers), 34.3, 33.7, 33.2, 33.1 (C4 major, minor and rotamers), 14.1, 14.0, 14.0, 13.9 (OCH₂CH₃ major, minor and rotamers); *m/z* (ESI⁻) 415 and 417 ([M-H]⁻, 100%); HRMS (ESI⁺) *m/z*: [M+Na]⁺ Calcd for C₁₅H₁₇O₅N₂BrNaS 438.9934 and 440.9913; Found 438.9929 and 440.9908.

(5*R*)-2-(5-Bromopyridin-2-yl)-1-(3-ethoxy-3-oxopropanoyl)-5-methoxycarbonyl-1,3-thiazolidine, 24c

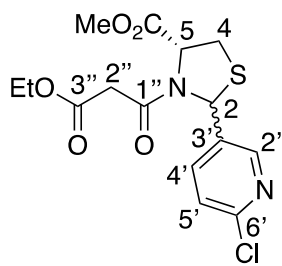


Yield (3.28 g, 85%); yellow oil; inseparable 1.3 : 1 *cis* and *trans* diastereomers; *R_f* = 0.30 (petrol : EtOAc; 1 : 1); $\nu_{\max}/\text{cm}^{-1}$ 1662 (s, C=O), 1739 (s, C=O); ¹H NMR (CDCl₃, 400 MHz): *cis* isomer as a 1 : 0.2 mixture of rotamers, *trans* isomer 1 : 0.9 as a mixture of

rotamers in CDCl₃: δ 8.51 (d, *J* = 2.5 Hz, 1H, H6' *trans* isomer major rotamer), 8.43 (d, *J* = 2.5 Hz, H6' *trans* isomer minor rotamer), 8.28 (d, *J* = 2.6 Hz, 1H, H6' *cis* isomer major rotamer), 8.24 (d, *J* = 2.3 Hz, H6' *cis* isomer minor rotamer), 8.03 (dd, *J* = 8.4, 2.7 Hz, 1H, H4' *trans* isomer major rotamer), 7.74 (dd, *J* = 8.3, 2.5 Hz, H4' *trans* isomer minor rotamer), 7.43 – 7.49 (m, 2H, H3' *trans* isomer minor and major rotamers, H4' *cis* isomer major and minor rotamers), 7.37 (d, *J* = 8.2 Hz, 1H, H3' *cis* isomer major rotamer), 7.22 (d, *J* = 8.2 Hz, H3' *cis* isomer minor rotamer), 6.21 (s, H2 *cis* isomer minor rotamer), 6.19 (s, 1H, H2 *cis* isomer major rotamer), 6.18 (s, H2 *trans* isomer minor rotamer), 6.15 (s, 1H, H2

trans isomer major rotamer), 5.24 (app d, $J = 6.3$ Hz, H5 *cis* isomer minor rotamer), 5.15 (app t, $J = 3.3$ Hz, 1H, H5 *cis* isomer major rotamer), 5.04 (dd, $J = 6.2, 2.6$ Hz, H5 *trans* isomer minor rotamer), 5.00 (app t, $J = 7.1$ Hz, 1H, H5 *trans* isomer major rotamer), 3.97 – 4.20 (m, 4H, OCH₂CH₃ *cis* and *trans* isomers), 3.80 (s, 3H, CO₂CH₃ *cis* isomer major rotamer), 3.78 (s, 3H, CO₂CH₃ *trans* isomer major rotamer), 3.77 (s, CO₂CH₃ *trans* isomer minor rotamer), 3.74 (s, CO₂CH₃ *cis* isomer minor rotamer), 3.09 – 3.49 (m, 8H, H_{4A}, H_{4B}, H_{2''A}, H_{2''B} *cis* and *trans* isomers), 1.14 – 1.26 (m, 6H, OCH₂CH₃ *cis* and *trans* isomers with rotamers); ¹³C NMR (CDCl₃, 101 MHz): δ 170.0, 170.0, 169.8, 169.0 (CO₂CH₃ major, minor and rotamers), 167.1, 166.8, 166.3, 165.9 (C3'' major, minor and rotamers), 165.2, 165.1, 165.0, 165.0 (C1'' major, minor and rotamers), 149.4, 148.4, 147.2, 146.8 (C2' major, minor and rotamers), 142.2, 142.0, 141.4, 140.7 (C6' major, minor and rotamers), 137.8, 137.3, 137.1, 137.1 (C4' major, minor and rotamers), 135.7, 135.5, 135.4, 134.1 (C3' major, minor and rotamers), 128.5, 128.3, 127.7, 127.6 (C5' major, minor and rotamers), 64.6, 64.2, 64.1, 63.9 (C5 major, minor and rotamers), 63.7, 63.3, 62.9, 62.3 (C2 major, minor and rotamers), 61.9, 61.8, 61.8, 61.7 (OCH₂CH₃ major, minor and rotamers), 53.5, 53.3, 52.9, 49.0 (CO₂CH₃ major, minor and rotamers), 43.0, 42.8, 42.3, 42.0 (C2'' major, minor and rotamers), 34.2, 33.7, 33.2, 32.1 (C4 major, minor and rotamers), 14.1, 14.0, 14.0, 14.0 (OCH₂CH₃ major, minor and rotamers); m/z (ESI⁺) 417 and 419 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z : [M+H]⁺ Calcd for C₁₅H₁₈O₅N₂BrS 417.0114 and 419.0094; Found 417.0109 and 419.0087.

(5R)-2-(6-Chloropyridin-3-yl)-1-(3-ethoxy-3-oxopropanoyl)-5-methoxycarbonyl-1,3-thiazolidine, 24d

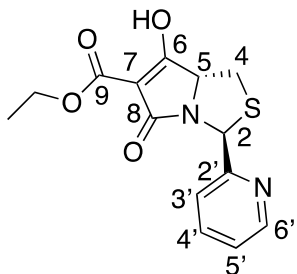


Yield (2.66 g, 77%); yellow oil; inseparable 1.2 : 1 *cis* and *trans* diastereomers; $R_f = 0.39$ (petrol : EtOAc; 1 : 1); $\nu_{\max}/\text{cm}^{-1}$ 1660 (s, C=O), 1737 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): *cis* isomer as a 1 : 0.5 mixture of rotamers, *trans* isomer 1 : 0.9 as a mixture of

rotamers in CDCl_3 : δ 8.53 (s, 1H, H2' *trans* isomer major rotamer), 8.46 (s, H2' *trans* isomer minor rotamer), 8.31 (s, 1H, H2' *cis* isomer major rotamer), 8.27 (s, H2' *cis* isomer minor rotamer), 8.13 – 8.19 (m, 1H, H4' *trans* isomer major rotamer), 7.85 (d, $J = 10.2$ Hz, H4' *trans* isomer minor rotamer), 7.58 (d, $J = 10.5$ Hz, 1H, H4' *cis* isomer major rotamer), 7.54 (d, $J = 10.5$ Hz, H4' *cis* isomer minor rotamer), 7.31 – 7.37 (m, 1H, H5' *trans* isomers with rotamers), 7.20 – 7.26 (m, 1H, H5' *cis* isomers with rotamers), 6.23 (s, 1H, H2 *cis* isomer with rotamers), 6.21 (s, 1H, H2, *trans* isomer major rotamer), 6.17 (s, H2 *trans* isomer minor rotamer), 5.26 (app d, $J = 6.7$ Hz, H5 *cis* isomer minor rotamer), 5.16 (app t, $J = 2.9$ Hz, 1H, H5 *cis* isomer major rotamer), 5.03 – 5.08 (m, 1H, H5 *trans* isomer major rotamer), 5.01 (app d, $J = 7.0$ Hz, H5 *trans* isomer minor rotamer), 4.00 – 4.22 (m, 4H, OCH_2CH_3 *cis* and *trans* isomers with rotamers), 3.82 (s, 3H, CO_2CH_3 *cis* isomer major rotamer), 3.80 (s, CO_2CH_3 *trans* isomer minor rotamer), 3.79 (s, 3H, CO_2CH_3 *trans* isomer major rotamer), 3.75 (s, CO_2CH_3 *cis* isomer minor rotamer), 3.09 – 3.53 (m, 8H, H4_A, H4_B, H2''_A, H2''_B *cis* and *trans* isomers with rotamers), 1.15 – 1.28 (m, 6H, OCH_2CH_3 *cis* and *trans* isomers with rotamers); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ 170.1, 170.1, 169.9, 169.1 (CO_2CH_3 major, minor and rotamers), 167.2, 166.9, 166.4, 166.0 (C3'' major, minor and rotamers), 166.0, 165.2, 165.0 (C1'' major, minor and rotamers), 151.8, 151.6, 150.9, 150.4 (C2' major, minor and rotamers), 149.1, 148.1, 146.8, 146.5 (C6' major, minor and

rotamers), 138.1, 137.4, 136.9, 136.8 (C4' major, minor and rotamers), 135.7, 135.7, 135.1, 133.7 (C3' major, minor and rotamers), 124.8, 124.7, 124.1, 123.9 (C5' major, minor and rotamers), 64.7, 64.3, 64.2, 64.0 (C5 major, minor and rotamers), 63.8, 63.4, 63.0, 62.3 (C2 major, minor and rotamers), 62.0, 62.0, 61.9, 61.8 (OCH₂CH₃ major, minor and rotamers), 53.6, 53.4, 53.0, 49.0 (CO₂CH₃ major, minor and rotamers), 43.2, 43.0, 42.4, 42.1 (C2'' major, minor and rotamers), 34.3, 34.0, 33.3, 32.2 (C4 major, minor and rotamers), 14.1, 14.1, 14.0, 14.0 (OCH₂CH₃ major, minor and rotamers); *m/z* (ESI⁺) 373 ([M+H]⁺, 100%) and 375 ([M+H]⁺, 33%); HRMS (ESI⁺) *m/z*: [M+H]⁺ Calcd for C₁₅H₁₈O₅N₂ClS 373.0620 and 375.0590; Found 373.0613 and 375.0582.

(2*S*,5*R*)-1-Aza-7-ethoxycarbonyl-6-hydroxy-8-oxo-2-(pyridin-2-yl)-3-thiabicyclo[3.3.0]oct-6-ene, 25a



Yield (870 mg, 48%); brown oil; *R_f* = 0.23 (MeOH : EtOAc; 1 : 6);

[α]_D²⁵ = -196.7 (*c* = 0.55, MeOH); *v*_{max}/cm⁻¹ 1589 (s, C=C), 1636 (s,

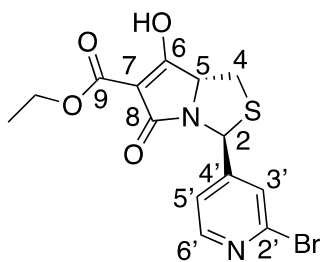
C=O); ¹H NMR (methanol-*d*₄, 500 MHz): δ (C6-OH not seen), 8.44

(d, *J* = 5.6 Hz, 1H, H6'), 7.78 (app td, *J* = 7.7, 1.8 Hz, 1H, H4'), 7.47

(d, *J* = 7.7 Hz, 1H, H3'), 7.27 (dd, *J* = 7.7, 5.6 Hz, 1H, H5'), 6.36 (s, 1H, H2), 4.40 (app t, *J* = 7.7 Hz, 1H, H5), 4.11 – 4.27 (m, 2H, OCH₂CH₃), 3.24 (dd, *J* = 10.9, 7.3 Hz, 1H, H4_B), 2.91 (dd, *J* = 10.9, 8.1 Hz, 1H, H4_A), 1.28 (t, *J* = 7.3 Hz, 3H, OCH₂CH₃); ¹³C NMR (methanol-*d*₄, 101 MHz): δ 194.9 (C6), 179.5 (C8), 168.1 (C9), 162.3 (C2'), 149.8 (C6'), 139.0 (C4'), 123.9 (C3'), 121.2 (C5'), 91.3 (C7), 71.3 (C5), 65.2 (C2), 60.2 (OCH₂CH₃), 34.6 (C4), 15.0 (OCH₂CH₃); *m/z* (ESI⁻) 305 ([M-H]⁻, 100%); HRMS (ESI⁺) *m/z*: [M+H]⁺ Calcd for C₁₄H₁₅O₄N₂S 307.0747; Found 307.0747.

(2*S*,5*R*)-1-Aza-2-(2-bromopyridin-4-yl)-7-ethoxycarbonyl-6-hydroxy-8-oxo-3-

thiabicyclo[3.3.0]oct-6-ene, 25b



Yield (1.20 g, 35%); orange foamy solid, m.p. 165 – 171 °C; $R_f =$

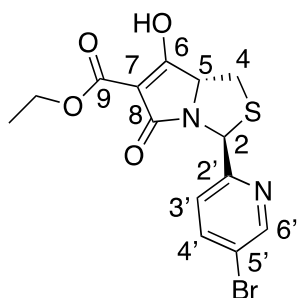
0.36 (MeOH : EtOAc; 1 : 6); $[\alpha]_D^{25} = -68.8$ ($c = 0.35$, CHCl_3);

$\nu_{\text{max}}/\text{cm}^{-1}$ (neat) 1586 (s, C=C), 1635 (s, C=O); ^1H NMR
(methanol- d_4 , 400 MHz): δ (C6-OH not seen), 8.27 (d, $J = 5.2$ Hz,

1H, H6'), 7.63 (s, 1H, H3'), 7.44 (d, $J = 5.2$ Hz, 1H, H5'), 6.30 (s, 1H, H2), 4.17 – 4.24 (m, 3H, OCH_2CH_3 and H5), 3.21 – 3.25 (obscured by solvent peak, 1H, H4_B), 2.97 (dd, $J = 11.1$, 7.2 Hz, 1H, H4_A), 1.26 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3); ^{13}C NMR (methanol- d_4 , 101 MHz): δ 194.6 (C6), 179.7 (C8), 167.7 (C9), 156.4 (C6'), 151.2 (C4'), 142.8 (C2'), 126.7 (C3'), 122.2 (C5'), 91.5 (C7), 70.2 (C5), 63.3 (C2), 60.2 (OCH_2CH_3), 34.6 (C4), 15.1 (OCH_2CH_3); m/z (ESI⁻) 383 and 385 ($[\text{M}-\text{H}]^-$, 100%); HRMS (ESI⁻) m/z : $[\text{M}-\text{H}]^-$ Calcd for $\text{C}_{14}\text{H}_{12}\text{O}_4\text{N}_2\text{BrS}$ 382.9707 and 384.9686; Found 382.9712 and 384.9690.

(2*S*,5*R*)-1-Aza-2-(5-bromopyridin-2-yl)-7-ethoxycarbonyl-6-hydroxy-8-oxo-3-

thiabicyclo[3.3.0]oct-6-ene, 25c



Yield (1.18 g, 40%); brown foamy solid, m.p. 157 – 163 °C; $R_f =$

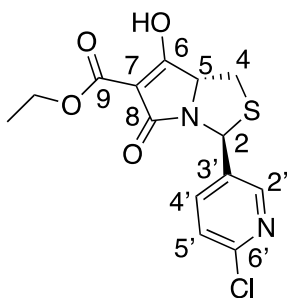
0.26 (MeOH : EtOAc; 1 : 6); $[\alpha]_D^{25} = -129.8$ ($c = 1.01$, CHCl_3);

$\nu_{\text{max}}/\text{cm}^{-1}$ (neat) 1581 (s, C=C), 1638 (s, C=O); ^1H NMR (CDCl_3 , 400
MHz): δ (C6-OH not seen), 8.60 (d, $J = 2.3$ Hz, 1H, H6'), 7.78 (dd, J

= 8.3, 2.3 Hz, 1H, H4'), 7.29 (d, $J = 8.3$ Hz, 1H, H3'), 6.29 (s, 1H, H2), 4.88 (app t, 1H, $J = 7.7$ Hz, H5), 4.36 (q, 2H, $J = 7.1$ Hz, OCH_2CH_3), 3.37 (dd, 1H, $J = 10.9$, 7.3 Hz, H4_B), 3.01 (dd, 1H, $J = 10.9$, 8.1 Hz, H4_A), 1.36 (t, 3H, $J = 7.1$ Hz, OCH_2CH_3); ^{13}C NMR (CDCl_3 , 101 MHz): δ 187.2 (C6), 170.0 (C8), 166.9 (C9), 158.4 (C2'), 150.7 (C6'), 139.5 (C4'), 121.5 (C3'), 119.8 (C5'),

97.8 (C7), 66.4 (C5), 62.6 (C2), 61.5 (OCH₂CH₃), 32.9 (C4), 14.3 (OCH₂CH₃); *m/z* (ESI⁻) 383 and 385 ([M-H]⁻, 100%); HRMS (ESI⁺) *m/z*: [M+Na]⁺ Calcd for C₁₄H₁₃O₄N₂BrNaS 406.9672 and 408.9651; Found 406.9673 and 408.9652.

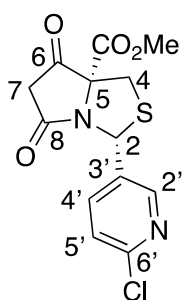
(2*S*,5*R*)-1-Aza-2-(6-chloropyridin-3-yl)-7-ethoxycarbonyl-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 25d



Yield (974 mg, 41%); orange oil; *R_f* = 0.36 (MeOH : EtOAc; 1 : 6); [α]_D²⁵ = -168.7 (*c* = 0.13, CHCl₃); *v*_{max}/cm⁻¹ 1585 (s, C=C), 1653 (s, C=O), 1704 (s, C=O); ¹H NMR (methanol-*d*₄, 400 MHz): δ (C6-OH not seen), 8.47 (s, 1H, H2'), 7.94 (d, *J* = 8.3 Hz, 1H, H4'), 7.45 (d, *J* = 8.3 Hz, 1H, H5'), 6.28 (s, 1H, H2), 4.83 (app t, *J* = 7.5 Hz, 1H, H5), 4.28 (q, *J* = 7.5 Hz, 2H, OCH₂CH₃), 3.32 – 3.38 (m, 1H, H4_B), 3.07 (dd, *J* = 10.9, 8.3 Hz, 1H, H4_A), 1.31 (t, *J* = 7.5 Hz, 3H, OCH₂CH₃); ¹³C NMR (methanol-*d*₄, 101 MHz): δ 185.5 (C6), 173.5 (C8), 164.7 (C9), 151.7 (C2'), 149.0 (C6'), 139.4 (C4'), 137.8 (C3'), 125.4 (C5'), 98.8 (C7), 67.6 (C5), 61.5 (C2), 60.9 (OCH₂CH₃), 33.7 (C4), 14.7 (OCH₂CH₃); *m/z* (ESI⁻) 339 ([M-H]⁻, 100%) and 341 ([M-H]⁻, 33%); HRMS (ESI⁻) *m/z*: [M-H]⁻ Calcd for C₁₄H₁₂O₄N₂ClS 339.0212 and 341.0182; Found 339.0208 and 341.0177.

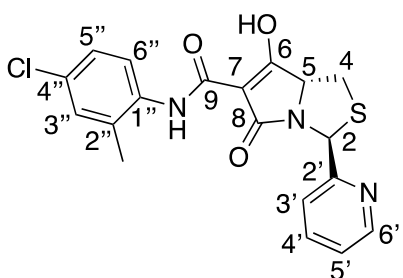
(2R,5R)-1-Aza-2-(6-chloropyridin-3-yl)-5-methoxycarbonyl-6,8-dioxo-3-

thiabicyclo[3.3.0]-octane, 26d



Yield (70 mg, 3%); orange oil; $R_f = 0.21$ (MeOH : EtOAc; 1 : 6); $[\alpha]_D^{25} = -113.9$ ($c = 1.00$, MeOH); $\nu_{\max}/\text{cm}^{-1}$ 1583 (s, C=C), 1695 (s, C=O); $^1\text{H NMR}$ (methanol- d_4 , 400 MHz): δ 8.48 (s, 1H, H2'), 7.95 (d, $J = 8.3$ Hz, 1H, H4'), 7.45 (d, $J = 8.3$ Hz, 1H, H5'), 6.40 (s, 1H, H2), 4.17 – 4.30 (m, 2H, H7), 3.70 (s, 3H, CO_2CH_3), 3.21 – 3.28 (obscured, 1H, H4_B), 3.03 (dd, 1H, $J = 11.2, 7.2$ Hz, H4_A); $^{13}\text{C NMR}$ (methanol- d_4 , 101 MHz): δ 194.6 (C6), 180.0 (C8), 167.1 (CO_2CH_3), 151.2 (C2'), 148.9 (C6'), 139.4 (C4'), 139.1 (C3'), 125.2 (C5'), 70.4 (C5), 62.5 (C2), 59.5 (CO_2CH_3), 50.3 (C7), 34.8 (C4); m/z (ESI⁻) 325 ([M-H]⁻, 100%) and 327 ([M-H]⁻, 33%); HRMS (ESI⁺) m/z : [M+H]⁺ Calcd for $\text{C}_{13}\text{H}_{12}\text{O}_4\text{N}_2\text{ClS}$ 327.0201 and 329.0171; Found 327.0203 and 329.0172.

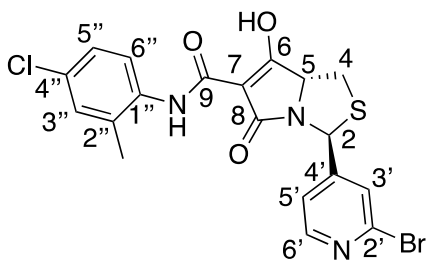
(2S,5R)-1-Aza-7-(4-chloro-2-methylphenylaminocarbonyl)-6-hydroxy-8-oxo-2-(pyridin-2-yl)-3-thiabicyclo[3.3.0]oct-6-ene, 27a



Yield (183 mg, 70%); brown oil; $R_f = 0.34$ (MeOH : EtOAc; 1 : 6); $[\alpha]_D^{25} = -114.6$ ($c = 0.67$, CHCl_3); $\nu_{\max}/\text{cm}^{-1}$ 1644 (s, C=O), 1678 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ (OH not observed), 9.39 (br. s, 1H, NH), 8.61 (br. d, 1H, H6'), 8.02 (br. d, 1H, H6''), 7.72 (t, $J = 7.7$ Hz, 1H, H4'), 7.41 (d, $J = 7.7$ Hz, 1H, H3'), 7.19 (br. s, 3H, H3'', H5'' and H5'), 6.35 (s, 1H, H2), 5.11 (br. s, 1H, H5), 3.45 (dd, $J = 18.7, 8.1$ Hz, 1H, H4_B), 3.06 – 3.16 (m, 1H, H4_A), 2.33 (s, 3H, CH_3); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ 188.0 (C6), (C8 not shown), 163.8 (C9), 159.1 (C2'), 149.7 (C6'), 138.2 (C4'), 133.7 (C2''), 130.5 (C4''), 130.1 (C1'', C3''), 126.8 (C5''), 123.2 (C3'), 122.8 (C6''), 120.2 (C5'), (C7 not shown), 67.1 (C5), 62.2 (C2), 32.4 (C4), 17.8 (CH_3); m/z (ESI⁻) 400 ([M-H]⁻, 100%) and 402 ([M-H]⁻, 33%);

HRMS (ESI⁺) *m/z*: [M+H]⁺ Calcd for C₁₉H₁₇O₃N₃ClS 402.0674 and 404.0644; Found 402.0674 and 404.0643.

(2*S*,5*R*)-1-Aza-2-(2-bromopyridin-4-yl)-7-(4-chloro-2-methylphenylaminocarbonyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 27b



Yield (130 mg, 52%); orange oil; *R*_f = 0.30 (100% EtOAc);

[α]_D²⁵ = -151.6 (*c* = 1.01, CHCl₃); ν_{\max} /cm⁻¹ 1587 (s, C=C),

1634 (s, C=O), 1695 (s, C=O); ¹H NMR (CDCl₃, 400 MHz):

δ (OH not observed), 9.28 (br. s, 1H, NH), 8.37 (d, *J* =

5.0 Hz, 1H, H6'), 8.00 (d, *J* = 8.6 Hz, 1H, H6''), 7.59 (s, 1H, H3'), 7.33 (d, *J* = 5.0 Hz, 1H, H5'),

7.19 (app d, *J* = 7.0 Hz, 2H, H3'', H5''), 6.18 (s, 1H, H2), 4.81 (app t, *J* = 6.9 Hz, 1H, H5), 3.29

– 3.34 (m, 1H, H4_B), 3.10 (dd, *J* = 11.2, 8.3 Hz, 1H, H4_A), 2.33 (s, 3H, CH₃); ¹³C NMR (CDCl₃,

101 MHz): δ 184.9 (C6), 172.3 (C8), 163.6 (C9), 151.9 (C6'), 150.6 (C4'), 142.9 (C2'), 133.3

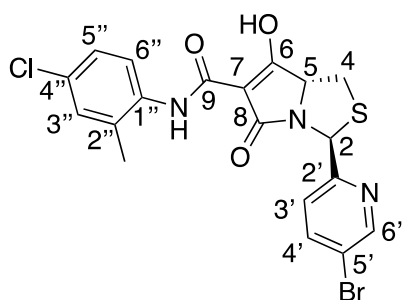
(C2''), 131.0 (C4''), 130.6 (C3''), 130.2 (C1''), 126.9 (C5''), 125.5 (C3'), 122.9 (C6''), 120.6

(C5'), 98.9 (C7), 66.5 (C5), 60.2 (C2), 32.6 (C4), 17.7 (CH₃); *m/z* (ESI⁻) 478, 480 ([M-H]⁻,

100%) and 482 ([M-H]⁻, 33%); HRMS (ESI⁻) *m/z*: [M-H]⁻ Calcd for C₁₉H₁₄O₃N₃BrClS 477.9633,

479.9613 and 481.9583; Found 477.9636, 479.9612 and 481.9580.

(2*S*,5*R*)-1-Aza-2-(5-bromopyridin-2-yl)-7-(4-chloro-2-methylphenylaminocarbonyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 27c



Yield (154 mg, 62%); orange oil; $R_f = 0.39$ (100% EtOAc);

$[\alpha]_D^{25} = -142.1$ ($c = 0.63$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1483 (s, C=C),

1587 (s, C=C), 1635 (s, C=O), 1693 (s, C=O); $^1\text{H NMR}$

(CDCl_3 , 400 MHz): δ 9.34 (br. s, 1H, NH), 8.65 (s, 1H, H6'),

8.03 (d, $J = 9.2$ Hz, 1H, H6''), 7.83 (d, $J = 8.3$ Hz, 1H, H4'), 7.56 (br. s, 1H, OH), 7.31 (d, $J =$

8.3 Hz, 1H, H3'), 7.19 (br. s, 2H, H3'', H5''), 6.28 (s, 1H, H2), 5.08 (app t, $J = 7.6$ Hz, 1H, H5),

3.44 (dd, $J = 10.8, 7.5$ Hz, 1H, H4_B), 3.10 (dd, $J = 10.8, 8.5$ Hz, 1H, H4_A), 2.33 (s, 3H, CH₃);

$^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ 185.0 (C6), 171.9 (C8), 163.7 (C9), 157.8 (C2'), 151.1 (C6'),

139.7 (C4'), 133.5 (C2''), 130.5 (C4''), 130.3 (C3''), 130.1 (C1''), 126.9 (C5''), 122.8 (C6''),

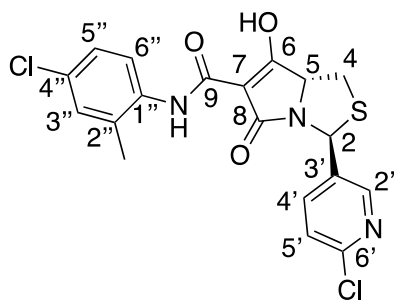
121.5 (C3'), 120.1 (C5'), 99.0 (C7), 67.0 (C5), 61.8 (C2), 32.3 (C4), 17.7 (CH₃); m/z (ESI⁻) 478

and 480 ([M-H]⁻, 100%) and 482 ([M-H]⁻, 33%); HRMS (ESI⁻) m/z : [M-H]⁻ Calcd for

$\text{C}_{19}\text{H}_{14}\text{O}_3\text{N}_3\text{BrClS}$ 477.9633, 479.9613 and 481.9583; Found 477.9632, 479.9608 and

481.9575.

(2*S*,5*R*)-1-Aza-7-(4-chloro-2-methylphenylaminocarbonyl)-2-(6-chloropyridin-3-yl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 27d



Yield (171 mg, 67%); orange oil; $R_f = 0.31$ (100% EtOAc);

$[\alpha]_D^{25} = -145.4$ ($c = 1.01$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1585 (s, C=C),

1633 (s, C=O), 1693 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ

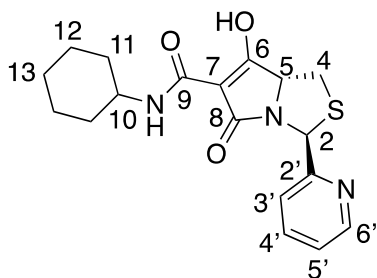
9.29 (br. s, 1H, NH), 8.90 (br. s, 1H, OH), 8.52 (s, 1H, H2'),

8.00 (d, $J = 8.9$ Hz, 1H, H6''), 7.78 (d, $J = 8.3$, 1H, H4'), 7.34 (d, $J = 8.3$ Hz, 1H, H5'), 7.19

(app d, $J = 7.0$ Hz, 2H, H3'', H5''), 6.24 (s, 1H, H2), 4.82 (app t, $J = 7.4$ Hz, 1H, H5), 3.33 (dd,

$J = 11.3, 7.1$ Hz, 1H, H_{4B}), 3.10 (dd, $J = 11.3, 8.4$ Hz, 1H, H_{4A}), 2.33 (s, 3H, CH₃); ¹³C NMR (CDCl₃, 101 MHz): δ 184.7 (C6), 172.3 (C8), 163.7 (C9), 151.7 (C2'), 148.3 (C6'), 137.6 (C4'), 134.8 (C3'), 133.5 (C2''), 130.7 (C4''), 130.5 (C3''), 130.3 (C1''), 127.0 (C5''), 124.5 (C5'), 123.0 (C6''), 99.2 (C7), 66.6 (C5), 59.5 (C2), 32.7 (C4), 17.8 (CH₃); m/z (ESI⁻) 434 ([M-H]⁻, 100%) and 436 ([M-H]⁻, 65%); HRMS (ESI⁻) m/z : [M-H]⁻ Calcd for C₁₉H₁₄O₃N₃Cl₂S 434.0138 and 436.0109; Found 434.0134 and 436.0104.

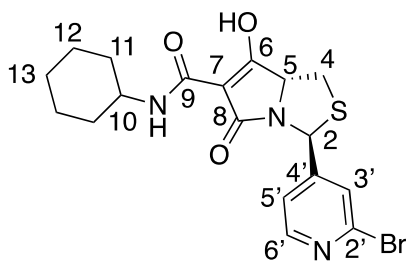
(2*S*,5*R*)-1-Aza-7-(cyclohexylaminocarbonyl)-6-hydroxy-8-oxo-2-(pyridin-2-yl)-3-thiabicyclo[3.3.0]oct-6-ene, 28a



Yield (96 mg, 41%); orange oil; $R_f = 0.47$ (MeOH : EtOAc; 1 : 6); $[\alpha]_D^{25} = +116.9$ ($c = 1.00$, CHCl₃); $\nu_{\max}/\text{cm}^{-1}$ 1557 (s, C=C), 1620 (s, C=O), 1688 (s, C=O); ¹H NMR (CDCl₃, 400 MHz): δ (NH and OH obscured), 8.58 (d, $J = 5.2$ Hz, 1H, H_{6'}), 7.67

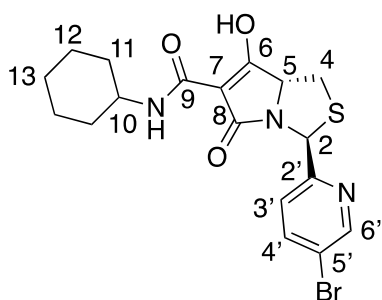
(app td, $J = 7.7, 1.7$ Hz, 1H, H_{4'}), 7.38 (d, $J = 7.7$ Hz, 1H, H_{3'}), 7.20 (dd, $J = 7.7, 5.2$ Hz, 1H, H_{5'}), 6.33 (s, 1H, H₂), 4.84 (br. s, 1H, H₅), 3.79 – 3.89 (m, 1H, H₁₀), 3.35 (dd, $J = 10.9, 7.3$ Hz, 1H, H_{4B}), 3.03 (dd, $J = 10.9, 8.4$ Hz, 1H, H_{4A}), 1.87 – 1.98 (m, 2H, 2 x H₁₁), 1.69 – 1.80 (m, 2H, 2 x H₁₂), 1.56 – 1.65 (m, 2H, 2 x H₁₃), 1.17 – 1.44 (m, 4H, 2 x H₁₁, 2 x H₁₂); ¹³C NMR (CDCl₃, 101 MHz): δ (C8 and C6 not shown), 165.5 (C9), 159.8 (C2'), 149.9 (C6'), 137.1 (C4'), 122.9 (C3'), 119.9 (C5'), 100.1 (C7), 68.3 (C5), 62.8 (C2), 46.0 (C10), 38.8 (C11), 32.8 (C4), 25.4 (C13), 24.6 (C12); m/z (ESI⁻) 358 ([M-H]⁻, 100%); HRMS (ESI⁺) m/z : [M+H]⁺ Calcd for C₁₈H₂₂O₃N₃S 360.1376; Found 360.1378.

(2*S*,5*R*)-1-Aza-2-(2-bromopyridin-4-yl)-7-(cyclohexylaminocarbonyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 28b



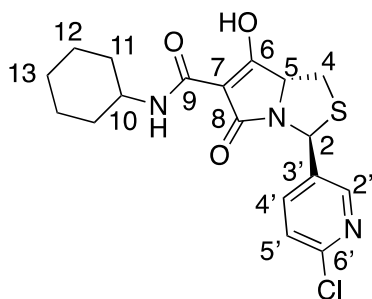
Yield (34 mg, 15%); orange oil; $R_f = 0.65$ (MeOH : EtOAc; 1 : 9); $[\alpha]_D^{25} = -128.0$ ($c = 0.60$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1586 (s, C=C), 1624 (s, C=O), 1691 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): a 3.3 : 1 mixture of AB/CD tautomers: δ 8.34 (d, $J = 5.0$ Hz, 1H, H6'), 7.86 (br. s, 1H, OH), 7.57 (s, 1H, H3'), 7.42 (br. s, 1H, NH), 7.31 (d, $J = 5.0$ Hz, 1H, H5'), 6.24 (s, H2 minor tautomer), 6.16 (s, 1H, H2 major tautomer), 4.73 (br. s, H5 minor tautomer), 4.61 (br. s, 1H, H5 major tautomer), 3.79 – 3.92 (m, 1H, H10), 3.21 – 3.32 (m, 1H, H4_B), 3.01 – 3.11 (m, 1H, H4_A), 1.88 – 1.97 (m, 2H, 2 x H11), 1.71 – 1.80 (m, 2H, 2 x H12), 1.55 – 1.68 (m, 2H, 2 x H13), 1.21 – 1.48 (m, 4H, 2 x H11, 2 x H12); $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 126 MHz): δ 191.2 (C6 minor), 189.1 (C6 major), 178.4 (C8 minor), 172.8 (C8 major), 165.4 (C9), 152.6 (C6'), 150.5 (C4'), 142.8 (C2'), 125.6 (C3'), 120.7 (C5'), 95.8 (C7 minor), 93.8 (C7 major), 70.5 (C5 minor), 67.6 (C5 major), 61.2 (C2 minor), 60.6 (C2 major), 49.9 (C10 minor), 48.6 (C10 major), 32.8 (C4), 32.1 (C11), 25.3 (C13), 24.5 (C12); m/z (ESI⁻) 436 and 438 ($[\text{M}-\text{H}]^-$, 100%); HRMS (ESI⁻) m/z : $[\text{M}-\text{H}]^-$ Calcd for $\text{C}_{18}\text{H}_{19}\text{O}_3\text{N}_3\text{BrS}$ 436.0336 and 438.0316; Found 436.0336 and 438.0315.

(2*S*,5*R*)-1-Aza-2-(5-bromopyridin-2-yl)-7-(cyclohexylaminocarbonyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 28c



Yield (71 mg, 31%); yellow oil; $R_f = 0.81$ (MeOH : EtOAc; 1 : 6); $[\alpha]_D^{25} = -87.3$ ($c = 1.01$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1558 (s, C=C), 1648 (s, C=O), 1690 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ 9.96 (br. s, 1H, NH), 8.60 (s, 1H, H6'), 7.77 (d, $J = 8.3$ Hz, 1H, H4'), 7.44 (br. s, 1H, OH), 7.28 (d, $J = 8.3$ Hz, 1H, H3'), 6.25 (s, 1H, H2), 4.81 (br. s, 1H, H5), 3.72 – 3.91 (m, 1H, H10), 3.32 (dd, $J = 10.9, 7.4$ Hz, 1H, H4_B), 3.01 (dd, $J = 10.9, 8.4$ Hz, 1H, H4_A), 1.78 – 2.09 (m, 2H, 2 x H11), 1.63 – 1.78 (m, 2H, 2 x H12), 1.48 – 1.65 (m, 2H, 2 x H13), 1.21 – 1.48 (m, 4H, 2 x H11, 2 x H12); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ 188.5 (C6), 172.3 (C8), 165.4 (C9), 158.4 (C2'), 150.8 (C6'), 139.5 (C4'), 121.3 (C3'), 119.7 (C5'), 107.3 (C7), 67.9 (C5), 62.2 (C2), 48.3 (C10), 32.7 (C4), 32.0 (C11), 25.3 (C13), 24.5 (C12); m/z (APCI⁺) 438 and 440 ($[\text{M}+\text{H}]^+$, 100%); HRMS (APCI⁺) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{18}\text{H}_{21}\text{O}_3\text{N}_3\text{BrS}$ 438.0482 and 440.0461; Found 438.0477 and 440.0456.

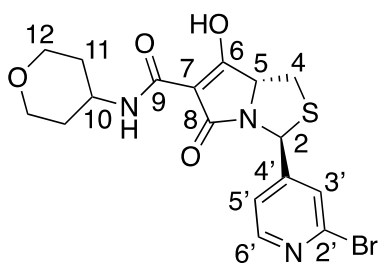
(2*S*,5*R*)-1-Aza-7-(cyclohexylaminocarbonyl)-2-(6-chloropyridin-3-yl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 28d



Yield (55 mg, 24%); yellow oil; $R_f = 0.62$ (MeOH : EtOAc; 1 : 6); $[\alpha]_D^{25} = -141.1$ ($c = 1.00$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1560 (s, C=C), 1624 (s, C=O), 1687 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ (NH obscured), 8.48 (s, 1H, H2'), 7.75 (d, $J = 8.3$ Hz, 1H, H4'), 7.30 (d, $J = 8.3$ Hz, 1H, H5'), 6.89 (br. s, 1H, OH), 6.24 (s, 1H, H2), 4.59 (br. s, 1H, H5), 3.76 – 3.90 (m, 1H, H10), 3.26 (dd, $J = 11.2, 7.4$ Hz, 1H, H4_B), 3.05 (dd, $J = 11.2, 8.2$ Hz, 1H, H4_A), 1.84 – 2.00 (m, 2H, 2 x H11), 1.69 – 1.80 (m, 2H, 2 x H12), 1.52 – 1.71 (m, 2H, 2 x

H13), 1.20 – 1.43 (m, 4H, 2 x H11, 2 x H12); ^{13}C NMR (CDCl_3 , 101 MHz): δ 188.7 (C6), 173.3 (C8), 165.4 (C9), 151.3 (C2'), 148.1 (C6'), 137.5 (C4'), 135.3 (C3'), 124.2 (C5'), 100.1 (C7), 67.4 (C5), 59.9 (C2), 48.5 (C10), 32.7 (C4), 32.0 (C11), 25.3 (C13), 24.5 (C12); m/z (ESI $^-$) 392 ($[\text{M}-\text{H}]^-$, 100%) and 394 ($[\text{M}-\text{H}]^-$, 33%); HRMS (ESI $^-$) m/z : $[\text{M}-\text{H}]^-$ Calcd for $\text{C}_{18}\text{H}_{19}\text{O}_3\text{N}_3\text{ClS}$ 392.0841 and 394.0812; Found 392.0843 and 394.0811.

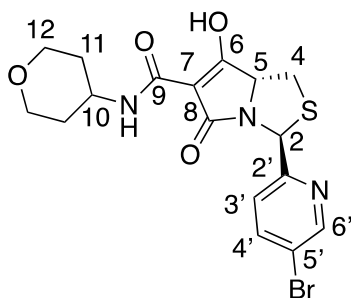
(2*S*,5*R*)-1-Aza-2-(2-bromopyridin-4-yl)-6-hydroxy-8-oxo-7-(tetrahydro-2*H*-pyran-4-aminocarbonyl)-3-thiabicyclo[3.3.0]oct-6-ene, 29b



Yield (28 mg, 12%); orange oil; R_f = 0.44 (MeOH : EtOAc; 1 : 6); $[\alpha]_D^{25} = -127.7$ ($c = 1.30$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1587 (s, C=C), 1623 (s, C=O), 1648 (s, C=O), 1691 (s, C=O); ^1H NMR (CDCl_3 , 400 MHz): a 3.3 : 1 mixture of AB/CD tautomers: δ 9.29 (br. s, 1H, NH), 8.53 (d, $J = 5.0$ Hz, H6' minor), 8.35 (d, $J = 5.1$ Hz, 1H, H6' major), 8.07 (s, H3' minor), 7.83 (d, $J = 5.0$ Hz, H5' minor), 7.57 (s, 1H, H3'), 7.41 (br. s, 1H, OH), 7.32 (d, $J = 5.1$ Hz, 1H, H5' major), 6.20 (s, H2 minor), 6.14 (s, 1H, H2 major), 4.67 (br. s, 1H, H5 major), 4.39 (dd, $J = 7.1, 2.2$ Hz, H5 minor), 4.03 – 4.11 (m, 1H, H10), 3.98 (d, $J = 11.3$ Hz, 2H, 2 x H12), 3.46 – 3.52 (m, 2H, 2 x H12), 3.27 (dd, $J = 11.2, 7.2$ Hz, 1H, H4_B), 3.05 (dd, $J = 11.2, 8.1$ Hz, 1H, H4_A), 1.92 (d, $J = 11.3$ Hz, 2H, 2x H11), 1.54 – 1.67 (m, 2H, 2x H11); $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 126 MHz): δ 193.0 (C6 minor), 187.2 (C6 major), 176.9 (C8 minor), 172.5 (C8 major), 165.9 (C9 minor), 165.3 (C9 major), 152.4 (C6' minor), 151.0 (C6' major), 150.5 (C4' major), 150.1 (C4' minor), 142.8 (C2' major), 140.5 (C2' minor), 128.3 (C3' minor), 125.6 (C3' major), 122.4 (C5' minor), 120.7 (C5' major), 95.7 (C7 major), 83.9 (C7 minor), 67.0 (C5), 66.4 (C12), 60.4 (C2), 45.7 (C10), 32.7 (C4), 32.0 (C11); m/z (ESI $^+$) 440 and 442

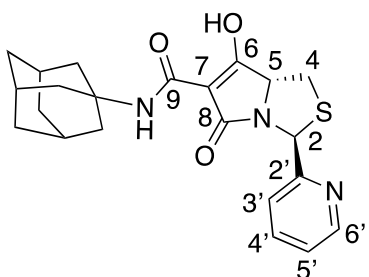
([M+H]⁺, 100%); HRMS (ESI⁻) *m/z*: [M-H]⁻ Calcd for C₁₇H₁₇O₄N₃BrS 438.0129 and 440.0108; Found 438.0134 and 440.0111.

(2*S*,5*R*)-1-Aza-2-(5-bromopyridin-2-yl)-6-hydroxy-8-oxo-7-(tetrahydro-2*H*-pyran-4-aminocarbonyl)-3-thiabicyclo[3.3.0]oct-6-ene, 29c



Yield (23 mg, 10%); orange oil; *R_f* = 0.50 (MeOH : EtOAc; 1 : 6); [α]_D²⁵ = -119.8 (*c* = 0.74, CHCl₃); *v*_{max}/cm⁻¹ 1578 (s, C=C), 1644 (s, C=O), 1676 (s, C=O); ¹H NMR (CDCl₃, 400 MHz): a 3.4 : 1 mixture of AB/CD tautomers: δ (NH and OH obscured), 8.63 (s, H6' minor), 8.61 (s, 1H, H6' major), 7.78 (d, *J* = 8.3 Hz, 1H, H4' major), 7.29 (d, *J* = 8.3 Hz, 1H, H3'), 6.30 (s, 1H, H2 major), 6.25 (s, H2 minor), 4.90 (app t, *J* = 7.6 Hz, 1H, H5), 4.37 (app q, *J* = 7.0 Hz, 1H, H10), 3.90 – 4.02 (m, 2H, 2 x H12), 3.42 – 3.54 (m, 2H, 2 x H12), 3.37 (dd, 1H, *J* = 10.9, 7.3 Hz, H4_B), 2.97 – 3.07 (m, 1H, H4_A), 1.82 – 1.98 (m, 2H, 2 x H11), 1.52 – 1.68 (m, 2H, 2 x H11); ¹³C{¹H} NMR (CDCl₃, 126 MHz): δ 177.9 (C6), 173.0 (C8), 167.9 (C9), 159.1 (C2' minor), 158.2 (C2' major), 150.9 (C6' major), 150.8 (C6' minor), 139.7 (C4'), 121.6 (C3' minor), 121.4 (C3' major), 120.7 (C5' minor), 119.9 (C5' major), 96.2 (C7), 66.9, 66.7, 66.5, 66.1 (C5 and C12, major and minor tautomers), 62.4 (C2), 45.9 (C10), 32.8 (C4), 32.1 (C11); *m/z* (ESI⁺) 440 and 442 ([M+H]⁺, 100%); HRMS (ESI⁻) *m/z*: [M-H]⁻ Calcd for C₁₇H₁₇O₄N₃BrS 438.0129 and 440.0108; Found 438.0132 and 440.0110.

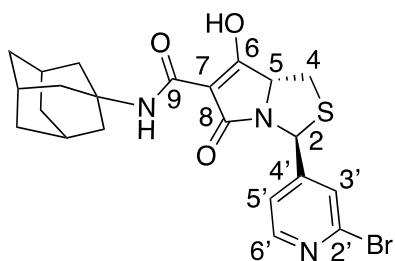
(2*S*,5*R*)-7-(Adamantylaminocarbonyl)-1-aza-6-hydroxy-8-oxo-2-(pyridin-2-yl)-3-thiabicyclo[3.3.0]oct-6-ene, 30a



Yield (118 mg, 44%); brown oil; $R_f = 0.70$ (MeOH : EtOAc; 1 : 6); $[\alpha]_D^{25} = -21.6$ ($c = 1.00$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1560 (s, C=C), 1633 (s, C=O), 1684 (C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ (OH obscured), 10.15 (br. s, 1H, NH), 8.58 (d, $J = 5.2$ Hz, 1H, H6'),

7.67 (app td, $J = 7.7, 1.7$ Hz, 1H, H4'), 7.38 (d, $J = 7.7$ Hz, 1H, H3'), 7.19 (dd, $J = 7.7, 5.2$ Hz, 1H, H5'), 6.31 (s, 1H, H2), 4.84 (br. s, 1H, H5), 3.33 (dd, $J = 10.9, 7.3$ Hz, 1H, H4_B), 3.01 (dd, $J = 10.9, 8.5$ Hz, 1H, H4_A), 2.11 (br. s, 3H, Adamantyl-CH), 2.05 (br. s, 6H, Adamantyl-CH₂), 1.69 (br. s, 6H, Adamantyl-CH₂); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ 188.8 (C6), (C8 not shown), 166.3 (C9), 159.8 (C2'), 149.8 (C6'), 137.1 (C4'), 122.9 (C3'), 119.8 (C5'), (C7 not shown), 68.0 (C5), 62.7 (C2), 53.3 (Adamantyl-C), 41.7 (Adamantyl-CH₂), 36.1 (Adamantyl-CH₂), 32.7 (C4), 29.4 (Adamantyl-CH); m/z (ESI⁻) 410 ([M-H]⁻, 100%); HRMS (ESI⁺) m/z : [M+H]⁺ Calcd for $\text{C}_{22}\text{H}_{26}\text{O}_3\text{N}_3\text{S}$ 412.1689; Found 412.1691.

(2*S*,5*R*)-7-(Adamantylaminocarbonyl)-1-aza-2-(2-bromopyridin-4-yl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 30b

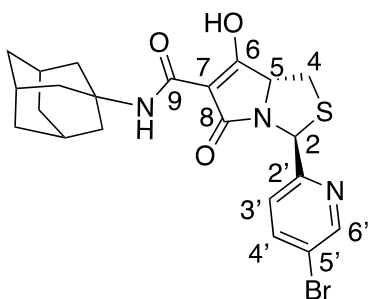


Yield (84 mg, 33%); orange oil; $R_f = 0.62$ (MeOH : EtOAc; 1 : 9); $[\alpha]_D^{25} = -135.6$ ($c = 1.01$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1584 (s, C=C), 1648 (s, C=O), 1688 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ (OH obscured), 8.99 (br. s, 1H, NH), 8.32 (d, $J =$

5.2 Hz, 1H, H6'), 7.55 (s, 1H, H3'), 7.30 (d, $J = 5.2$ Hz, 1H, H5'), 6.17 (s, 1H, H2), 4.48 (br. s, 1H, H5), 3.24 (dd, $J = 11.2, 7.4$ Hz, 1H, H4_B), 3.04 (dd, $J = 11.2, 8.1$ Hz, 1H, H4_A), 2.12 (br. s, 3H, Adamantyl-CH), 2.05 (br. s, 6H, Adamantyl-CH₂), 1.69 (br. s, 6H, Adamantyl-CH₂); ^{13}C

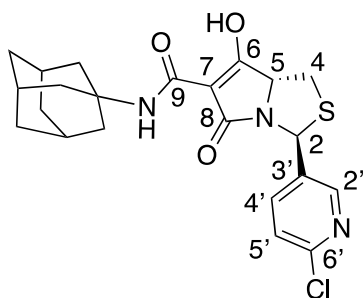
NMR (CDCl₃, 101 MHz): δ 190.0 (C6), C8 not shown, 166.2 (C9), 152.8 (C6'), 150.4 (C4'), 142.8 (C2'), 125.5 (C3'), 120.7 (C5'), 100.1 (C7), 68.4 (C5), 60.8 (C2), 53.9 (Adamantyl-C), 41.7 (Adamantyl-CH₂), 36.1 (Adamantyl-CH₂), 32.9 (C4), 29.4 (Adamantyl-CH); *m/z* (ESI⁻) 488 and 490 ([M-H]⁻, 100%); HRMS (ESI⁻) *m/z*: [M-H]⁻ Calcd for C₂₂H₂₃O₃N₃BrS 488.0649 and 490.0629; Found 488.0652 and 490.0629.

(2*S*,5*R*)-7-(Adamantylaminocarbonyl)-1-aza-2-(5-bromopyridin-2-yl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 30c



Yield (97 mg, 38%); brown oil; *R_f* = 0.56 (MeOH : EtOAc; 1 : 9); $[\alpha]_D^{25}$ = -97.0 (*c* = 0.53, CHCl₃); $\nu_{\max}/\text{cm}^{-1}$ 1559 (s, C=C), 1648 (s, C=O), 1687 (s, C=O); ¹H NMR (CDCl₃, 400 MHz): a 2.3 : 1 mixture of AB/CD tautomers: δ 8.63 (s, 1H, H6'), 7.80 (dd, *J* = 8.3, 2.2 Hz, 1H, H4'), 7.30 (d, *J* = 8.3 Hz, 1H, H3'), 6.53 (br. s, 2H, OH and NH), 6.33 (s, H2 minor), 6.25 (s, 1H, H2 major), 4.81 (app t, *J* = 7.8 Hz, 1H, H5 major), 4.57 (app t, *J* = 7.9 Hz, H5 minor), 3.33 (app dt, 1H, *J* = 11.0, 6.8 Hz, H4_B), 2.99 – 3.07 (m, 1H, H4_A), 2.12 (br. s, 3H, Adamantyl-CH), 2.05 (br. s, 6H, Adamantyl-CH₂), 1.70 (br. s, 6H, Adamantyl-CH₂); ¹³C NMR (CDCl₃, 101 MHz): δ 189.2 (C6 major), 172.5 (C8 major), 166.2 (C9 major), 158.4 (C2'), 150.9 (C6'), 140.9 (C4' minor), 139.6 (C4' major), 121.3 (C3'), 119.8 (C5'), 94.3 (C7 major), 68.1 (C5 major), 66.3 (C5 minor), 62.9 (C2 minor), 62.2 (C2 major), 53.4 (Adamantyl-C), 44.3 (Adamantyl-CH₂ minor), 41.7 (Adamantyl-CH₂ major), 36.2 (Adamantyl-CH₂ major), 36.0 (Adamantyl-CH₂ minor), 33.0 (C4 minor), 32.6 (C4 major), 29.8 (Adamantyl-CH minor), 29.4 (Adamantyl-CH major); *m/z* (ESI⁺) 490 and 492 ([M+H]⁺, 100%); HRMS (ESI⁺) *m/z*: [M+H]⁺ Calcd for C₂₂H₂₅O₃N₃BrS 490.0795 and 492.0774; Found 490.0795 and 492.0773.

(2*S*,5*R*)-7-(Adamantylaminocarbonyl)-1-aza-2-(6-chloropyridin-3-yl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 30d



Yield (92 mg, 35%); orange oil; $R_f = 0.70$ (MeOH : EtOAc; 1 : 9); $[\alpha]_D^{25} = -90.5$ ($c = 1.10$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1562 (s, C=C), 1627 (s, C=O), 1686 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 500 MHz): a 3.2 : 1 mixture of AB/CD tautomers: δ (NH obscured), 8.49 (s, 1H, H2'), 7.75 (d, $J = 8.3$, 1H, H5' major), 7.39 (br. s, 1H, OH), 7.32 (d, $J = 8.3$ Hz, 1H, H4' major tautomer), 6.31 (s, H2 minor), 6.23 (s, 1H, H2 major), 4.59 (app t, $J = 7.7$ Hz, 1H, H5), 3.26 (dd, $J = 11.2, 7.2$ Hz, 1H, H4_B), 3.05 (dd, $J = 11.2, 8.2$ Hz, 1H, H4_A), 2.13 (br. s, 3H, Adamantyl-CH), 2.06 (br. s, 6H, Adamantyl-CH₂), 1.70 (br. s, 6H, Adamantyl-CH₂); $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 126 MHz): δ 191.1 (C6 minor), 189.5 (C6 major), 173.0 (C8 major), 167.9 (C9 minor), 166.2 (C9 major), 151.3 (C2'), 148.2 (C6'), 137.5 (C4'), 135.3 (C3'), 124.2 (C5'), 93.9 (C7 major), 67.7 (C5), 59.9 (C2), 53.7 (Adamantyl-C), 41.7 (Adamantyl-CH₂), 36.2 (Adamantyl-CH₂ major), 36.0 (Adamantyl-CH₂ minor), 32.8, 32.1 (C4 major and minor tautomers), 29.8 (Adamantyl-CH minor), 29.4 (Adamantyl-CH major); m/z (ESI⁻) 444 ([M-H]⁻, 100%) and 446 ([M-H]⁻, 33%); HRMS (ESI⁻) m/z : [M-H]⁻ Calcd for $\text{C}_{22}\text{H}_{23}\text{O}_3\text{N}_3\text{ClS}$ 444.1154 and 446.1125; Found 444.1157 and 446.1125.

6.3 Experimental for Chapter 3

Compounds **23e**, **24e**, **25e**, **27e** and **30e** have been published in the literature.^{109,110} These compounds were synthesised according to the literature published procedures (general procedures **A-D**, section 6.2) and used as starting materials for the relevant reactions in this chapter.

Compound **33** were synthesised according to general procedure **B** (section 6.2.2). Compounds **32**, **35** and **44e** were synthesised according to general procedure **C** (section 6.2.3). Compounds **48-50c** were synthesised based on general procedure **D** (section 6.2.4).

6.3.1 Alternative procedure (E) for synthesis of *N*-acylthiazolidine **34**

T₃P (1.2 eqv., 50% w/v in DMF) was added dropwise to a stirred solution of pyridylacetic acid HCl (1.2 eqv.), DIPEA (5 eqv.) and thiazolidine (1.0 eqv., 1 mmol) in dry DMF (*c* = 0.2) at 0 °C. The mixture was stirred at 0 °C for 15 min and then at room temperature for 18 h. The reaction mixture was diluted with EtOAc and washed with distilled water and brine. The organic layers were separated, combined, dried with MgSO₄ and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography to give *N*-acylthiazolidine **34**.

6.3.2 General procedure (F) for synthesis of tetramate enol ethers **36-41**

Diethyl azodicarboxylate (DEAD, 1.4 eqv.) was added dropwise to a cooled solution of alcohol (1.2 eqv.), triphenylphosphine (PPh₃, 1.2 eqv.) and tetramate (1.0 eqv., 0.2 mmol) in dry THF (*c* = 0.1) at 0 °C. The mixture was stirred at 0 °C for 15 min and then at room

temperature for 20 h. It was then concentrated *in vacuo* and residue was purified by flash column chromatography to produce tetramate analogues **36-41**.

6.3.3 Synthesis of side chain C-methylated *N*-acylthiazolidine **43e** (Procedure G)

MeI (1.3 eqv.) was added dropwise to a stirred solution of NaH (1.3 eqv., 60% w/w oil suspension) and *N*-acylthiazolidine **24e** (1 eqv., 1 g, 3 mmol) in dry THF ($c = 0.03$) at 0 °C. The mixture was cooled to room temperature and stirred for 16 h. It was then neutralised with sat aq. NH₄Cl and extracted with EtOAc. The organic layer was separated, dried with MgSO₄ and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography to give **43e**.

6.3.4 General procedure (H) for synthesis of **45e** and **55e** by acylation

Ethyl chloroformate (1.2 eqv.) was added dropwise to a stirred solution of dry Et₃N or DMAP (1.2 eqv.) and the respective tetramate (1 eqv.) in dry DCM ($c = 0.1$) at 0 °C. The mixture was warmed to room temperature and stirred for 18 h. The reaction mixture was then diluted with DCM and washed with 2M HCl solution. The combined organic layers were dried with MgSO₄ and concentrated *in vacuo* and resulting residue was purified by flash column chromatography to give the desired product. Compounds **45e** and **55e** were synthesised based on this procedure from **44e** and **30e** respectively.

6.3.5 General procedure (I) for synthesis of THP-protected tetramate esters **47b-d**

A mixture of the tetramate (1 eqv., 100 mg), 3,4-dihydro-2H-pyran (1.2 eqv.) and catalytic *p*-TsOH monohydrate (0.03 eqv.) in DCM ($c = 0.1$) was stirred at room temperature for 16 h. The mixture was diluted with DCM and washed sequentially with sat. aq. NaHCO₃ and

brine. The organic layer was separated, dried with MgSO_4 and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography to yield THP-protected tetramate esters **47b-d**.

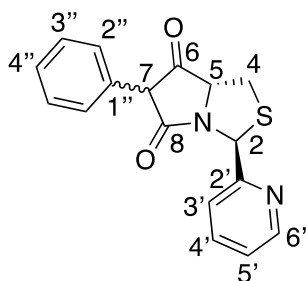
6.3.6 Synthesis of tetramate carboxamide **54e** with chloroacetyl chloride (Procedure J)

Chloroacetyl chloride (1.2 eqv.) was added dropwise to a stirred solution of dry Et_3N (1.2 eqv.) and tetramate carboxamide **30e** (0.2 mmol) in dry DCM ($c = 0.1$) at 0°C . The mixture was warmed to room temperature and stirred for 18 h. The reaction mixture was then concentrated *in vacuo* and resulting residue was purified by flash column chromatography to give the desired product **54e**.

6.3.7 Synthesis of tetramate carboxamide **56e** by transesterification (Procedure K)

A solution of benzyl alcohol (1.5 eqv.) and **55e** (1 eqv., 0.1 mmol) in toluene ($c = 0.05$) was heated under reflux for 48 h. The solvent was removed *in vacuo* and resulting residue purified by flash column chromatography to give the desired product **56e**.

(2*S*,5*R*)-1-aza-6,8-dioxo-7-phenyl-2-(pyridin-2-yl)-3-thiabicyclo[3.3.0]octane, **32**



Synthesised from **33** (220 mg, 0.64 mmol) by general procedure

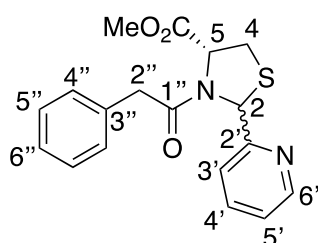
C; yield (70 mg, 35%); brown oil; $R_f = 0.25$ (100% EtOAc); ^1H

NMR (CDCl_3 , 400 MHz): a 7 : 3 mixture of keto/enol tautomers, with the keto form as a 2.6 : 1 $7R/S$ mixture of diastereomers: δ

8.33 (d, $J = 4.7$ Hz, 1H, H6'), 7.74 (app d, $J = 7.6$ Hz, 2H, H3' and H4'), 7.66 (app t, $J = 7.7$ Hz, 1H, H5'), 7.46 (d, $J = 7.8$ Hz, 1H, H4''), 7.28 (d, $J = 7.3$ Hz, 2H, H3''), 7.16 (d, $J = 7.2$ Hz, 2H, H2''), 6.25 (s, 1H, H2), 4.80 (dd, $J = 8.7, 6.6$ Hz, 1H, H5), 3.62 (s, H7 minor), 3.56 (s, 1H, H7

major), 3.20 (dd, $J = 10.8, 6.3$ Hz, 1H, H_{4B}), 2.88 (q, $J = 10.8$ Hz, 1H, H_{4A}); ¹³C NMR (CDCl₃, 101 MHz): δ (C6 not shown), 176.1 (C8), 159.4 (C2'), 148.3 (C6'), 138.4 (C4'), 130.7 (C1''), 129.5, 129.3 (C3'' major and minor), 128.3, 127.9 (C4'' major and minor), 127.8, 126.9 (C2'' major and minor), 123.3 (C3'), 121.1 (C5'), 104.8 (C7, enol), 67.3 (C5), 62.1 (C2), 43.2 (C7, keto), 33.7 (C4); m/z (ESI⁺) 311 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for C₁₇H₁₅O₂N₂S [M+H]⁺ 311.0896; found 311.0852.

(5R)-5-methoxycarbonyl-1-(2-phenylacetyl)-2-(pyridin-2-yl)-1,3-thiazolidine, 33

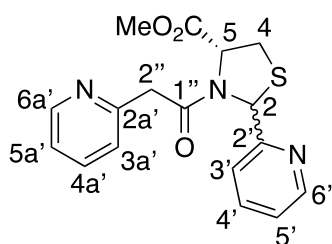


Synthesised from **23a** (200 mg, 0.9 mmol) by general procedure **B**; yield (221 mg, 75%); yellow oil; inseparable 2 : 1 *cis* and *trans* diastereomers; $R_f = 0.23$ (petrol : EtOAc; 3 : 2); ¹H NMR (CDCl₃, 400 MHz): *cis* as a 1 : 0.1 mixture of rotamers in

CDCl₃, *trans* as a 1 : 0.7 mixture of rotamers in CDCl₃: δ 8.53 (d, $J = 4.8$ Hz, 1H, H_{6'} *cis* major rotamer), 8.05 (d, $J = 7.9$ Hz, 1H, H_{3'} *cis* major rotamer), 7.66 (td, $J = 7.7, 1.7$ Hz, 1H, H_{4'} *cis* major rotamer), 7.56 – 7.66 (m, H_{4'} *trans* major and minor rotamers), 7.21 – 7.30 (m, 2 x H_{5''}, H_{6''} and H_{5'}, *trans* major and minor rotamers), 7.14 – 7.23 (m, 4H, 2 x H_{5''}, H_{6''} and H_{5'}, *cis* major rotamers), 7.03 (d, $J = 6.5$ Hz, 2H, H_{4''}, *cis* major rotamer), 6.44 (s, H₂, *cis* minor rotamer), 6.21 (s, 1H, H₂, *cis* major rotamer), 5.79 (s, H₂, *trans* minor rotamer), 5.60 (s, H₂, *trans* major rotamer), 4.92 – 4.96 (m, H₅, *cis* minor rotamer), 4.89 (dd, $J = 9.4, 6.5$ Hz, 1H, H₅, *cis* major rotamer), 4.49 (dd, $J = 6.4, 4.6$ Hz, H₅, *trans* major rotamer), 3.99 (dd, $J = 9.7, 6.7$ Hz, H₅, *trans* minor rotamer), 3.78 (s, 3H, CO₂CH₃, *cis* major rotamer), 3.77 (s, CO₂CH₃, *trans* major rotamer), 3.75 (s, CO₂CH₃, *trans* minor rotamer), 3.73 (s, CO₂CH₃, *cis* minor rotamer), 3.59 (s, H_{2''}, *trans* and minor major rotamer), 3.48 (d, $J = 9.6$ Hz, 2H, H_{2''}, *cis* major and minor rotamer), 3.38 – 3.44 (m, H_{4B}, *trans* major and

minor rotamers), 3.30 (dd, $J = 5.5, 3.8$ Hz, H_{4A}, *trans* major rotamer), 3.21 (dd, $J = 12.0, 6.5$ Hz, 1H, H_{4B}, *cis* major and minor rotamer), 3.12 (dd, $J = 12.0, 9.4$ Hz, 1H, H_{4A}, *cis* major and minor rotamer), 3.02 – 3.09 (m, H_{4A}, *trans* minor rotamers); ¹³C NMR (CDCl₃, 101 MHz): δ 175.1, 171.0, 170.5 (CO₂CH₃ major, minor and rotamers), 160.0 (C1''), 157.4, 156.7 (C2' major and minor isomers), 149.9, 149.6, 149.5 (C6' major, minor and rotamers), 137.8, 137.0, 137.0 (C4' major, minor and rotamers), 134.4, 133.3 (C3'' major and minor), 129.5, 129.1 (C5'' major, minor and rotamers), 128.7, 128.6 (C6'' major, minor and rotamers), 127.3, 127.1, 127.1 (C4'' major, minor and rotamers), 123.5, 123.2, 123.2 (C3' major, minor and rotamers), 122.2, 121.7, 120.6 (C5' major, minor and rotamers), 71.5, 71.0, 67.2 (C5 major, minor and rotamers), 66.3, 65.7, 65.2 (C2 major, minor and rotamers), 53.2, 52.9, 52.7 (CO₂CH₃ major, minor and rotamers), 41.7, 41.5, 39.4, 38.8 (C2'' major, minor and rotamers), 33.9, 31.4 (C4 *cis* and *trans*); m/z (ESI⁺) 343 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for C₁₈H₁₉O₃N₂S [M+H]⁺ 343.1111; found 343.1106.

(5R)-5-methoxycarbonyl-1-(2-pyridylacetyl)-2-(pyridin-2-yl)-1,3-thiazolidine, 34

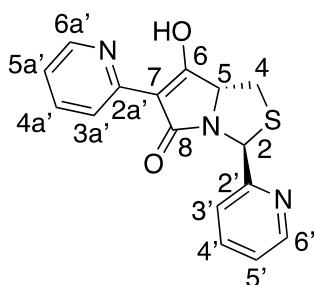


Synthesised from **23a** (220 mg, 1 mmol) by procedure E; yield (212 mg, 62%); greenish oil; inseparable 1.3 : 1 *cis* and *trans* diastereomers; $R_f = 0.51$ (petrol : EtOAc; 6 : 1); ¹H NMR (CDCl₃, 400 MHz): *cis* as a 1 : 0.2 mixture of rotamers in CDCl₃, *trans*

as a 1 : 0.7 mixture of rotamers in CDCl₃: δ 8.39 – 8.61 (m, 6H, H6' and H6a'), 8.10 (d, $J = 7.9$ Hz, 6H, H3' and H3a'), 7.46 – 7.76 (m, 6H, H4' and H4a'), 7.04 – 7.35 (m, 6H, H5' and H5a'), 6.58 (s, 1H, H2, *cis* major rotamer), 6.40 (s, H2, *cis* minor rotamer), 6.33 (s, 1H, H2, *trans* major rotamer), 6.23 (s, 1H, H2, *trans* minor rotamer), 5.64 (dd, $J = 6.6, 3.1$ Hz, H5, *cis* minor rotamer), 5.53 (app d, $J = 5.9$ Hz, 1H, H5, *trans* minor rotamer), 5.29 (app d, $J =$

7.9 Hz, 1H, H5, *trans* major rotamer), 4.94 (dd, $J = 9.3, 6.6$ Hz, 1H, H5, *cis* major rotamer), 3.80 (s, 3H, CO₂CH₃, *trans* major rotamer), 3.80 (s, 3H, CO₂CH₃, *cis* major rotamer), 3.77 (s, 3H, CO₂CH₃, *trans* minor rotamer), 3.76 (s, CO₂CH₃, *cis* minor rotamer), 3.72 (s, 2H, H2''), *cis* major rotamer), 3.60 (s, 2H, H2'', *trans* major rotamer), 3.56 (d, $J = 2.1$ Hz, 2H, H2'', *trans* minor rotamer), 3.52 (s, H2'', *cis* minor rotamer), 3.34 – 3.42 (m, 2H, H4_B *trans*, major and minor rotamers), 3.26 (dd, $J = 11.9, 6.6$ Hz, 1H, H4_B *cis*, major and minor rotamers), 3.11 – 3.21 (m, 3H, H4_A, major, minor and rotamers); ¹³C NMR (CDCl₃, 101 MHz): δ 170.9, 170.8, 170.1 (CO₂CH₃ major, minor and rotamers), 169.7, 169.0, 169.0 (C1'' major, minor and rotamers), 160.7, 160.5, 160.0 (C2' major and minor isomers), 155.0, 154.5, 154.4 (C2a' major and minor isomers), 150.0, 149.8, 149.5, 149.4, 149.2, 149.1 (C6' and C6a' major, minor and rotamers), 137.5, 137.3, 137.0, 136.8, 136.8, 136.7 (C4' and C4a' major, minor and rotamers), 124.3, 124.0, 123.8 (C3' major, minor and rotamers), 123.1, 122.5, 122.2 (C3a' major, minor and rotamers), 120.5, 119.8, 119.6 (C5' and C5a' major, minor and rotamers), 67.8, 67.4, 66.4, 65.9 (C5 major, minor and rotamers), 65.0, 64.2, 64.0, 63.8 (C2 major, minor and rotamers), 53.3, 53.1, 52.8 (CO₂CH₃ major, minor and rotamers), 45.5, 44.8, 44.3 (C2'' major, minor and rotamers), 31.6, 31.5, 31.4 (C4 major, minor and rotamers); m/z (ESI⁺) 344 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for C₁₇H₁₈O₃N₃S [M+H]⁺ 344.1063; found 344.1064.

(2*S*,5*R*)-1-aza-8-oxo-2,7-di(pyridin-2-yl)-3-thiabicyclo[3.3.0]oct-6-ene, 35



Synthesised from **34** (210 mg, 0.6 mmol) by general procedure

C; yield (80 mg, 42%); yellow oil; $R_f = 0.43$ (EtOAc : MeOH; 6 : 1);

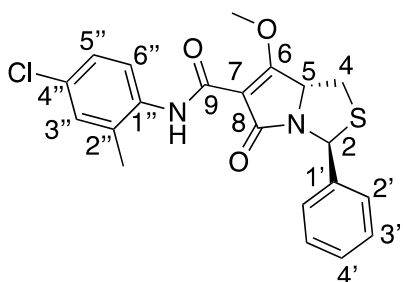
$\nu_{\max}/\text{cm}^{-1}$ 1583 (s, C=C), 1631 (s, C=O), 1670 (s, C=O); $^1\text{H NMR}$

(CDCl_3 , 400 MHz): 1 : 0.7 AB/CD tautomers: δ (C6-OH not

observed), 8.60 (d, $J = 4.4$ Hz, 2H, H6' and H6a', major tautomer), 8.45 (d, $J = 8.9$ Hz, 2H, H6' and H6a', minor tautomer), 7.80 – 7.91 (m, 4H, H4' and H4a' minor and major tautomers), 7.64 – 7.72 (m, 2H, H3' and H3a' major tautomer), 7.45 (d, $J = 7.8$ Hz, 2H, H3' and H3a' minor tautomer), 7.19 (d, $J = 5.2$ Hz, 2H, H5' and H5a' major tautomer), 7.02 (t, $J = 6.5$ Hz, 1H, H5' minor tautomer), 6.92 (br. s, 1H, H5a' minor tautomer), 6.54 (s, 1H, H2 major tautomer), 6.49 (s, 1H, H2 minor tautomer), 4.66 (app t 1H, $J = 7.8$ Hz, H5, major tautomer), 4.56 (app t, 1H, $J = 7.7$ Hz, H5, minor tautomer), 3.37 (dd, $J = 10.8, 7.3$ Hz, 2H, H4_B, major and minor tautomer), 3.08 (q, $J = 10.4$ Hz, 2H, H4_A, major and minor tautomer); $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 126 MHz): δ 194.3 (C6 major tautomer), 191.4 (C6 minor tautomer), 178.3 (C8 minor tautomer), 174.0 (C8 major tautomer), 160.8 (C2'), 149.7 (C6' and C6a'), 141.8 (C2a'), 137.0 (C4'), 134.7 (C4a'), 122.6 (C3' and C3a'), 119.8 (C5' and C5a'), 91.0 (C7 minor tautomer), 86.5 (C7 major tautomer), 71.0 (C5 minor tautomer), 69.9 (C5 major tautomer), 64.2 (C2), 33.9 (C4); m/z (ESI⁺) 312 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for $\text{C}_{16}\text{H}_{14}\text{O}_2\text{N}_3\text{S}$ [M+H]⁺ 312.0801; found 312.0802.

Compounds **36-41** were synthesised from the respective tetramate carboxamides **27/30** (0.2 mmol) by general procedure **F**.

(2*S*,5*R*)-1-Aza-7-(4-chloro-2-methylphenylaminocarbonyl)-6-methoxy-8-oxo-2-phenyl-3-thiabicyclo[3.3.0]oct-6-ene, 36e



Yield (25 mg, 30%); yellow oil; $R_f = 0.43$ (petrol : EtOAc;

1 : 4); $[\alpha]_D^{25} = -114.0$ ($c = 1.0$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1585 (s,

C=C), 1618 (s, C=O), 1694 (s, C=O), 3258 (br, N-H); ^1H

NMR (CDCl_3 , 400 MHz): δ 10.29 (s, 1H, NH), 8.15 (d, $J =$

9.4 Hz, 1H, H6''), 7.47 (d, $J = 7.4$ Hz, 2H, H2'), 7.38 (t, $J = 7.3$ Hz, 2H, H3'), 7.32 (t, $J = 7.2$ Hz,

1H, H4'), 7.12 – 7.19 (m, 2H, H3'', H5''), 6.33 (s, 1H, H2), 4.88 (dd, $J = 9.0, 6.5$ Hz, 1H, H5),

4.32 (s, 3H, OCH_3), 3.36 (dd, $J = 10.9, 6.5$ Hz, 1H, H4_B), 3.02 (dd, $J = 10.8, 9.1$ Hz, 1H, H4_A),

2.33 (s, 3H, CH_3); ^{13}C NMR (CDCl_3 , 101 MHz): δ 178.8 (C6), 173.3 (C8), 158.7 (C9), 139.7

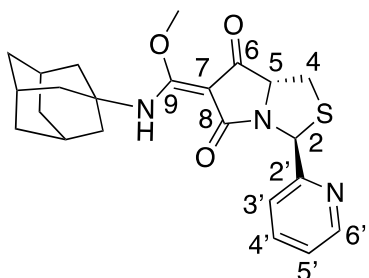
(C1'), 135.1 (C2''), 130.2 (C4''), 130.0 (C3''), 129.0 (C1''), 128.9 (C3'), 128.5 (C4'), 126.6

(C5''), 126.4 (C2'), 122.7 (C6''), 102.4 (C7), 65.5 (C5), 62.0 (OCH_3), 61.5 (C2), 33.5 (C4), 18.1

(CH_3); m/z (ESI⁺) 415 ($[\text{M}+\text{H}]^+$, 100%) and 417 ($[\text{M}+\text{H}]^+$, 33%); HRMS (ESI⁺) m/z calcd for

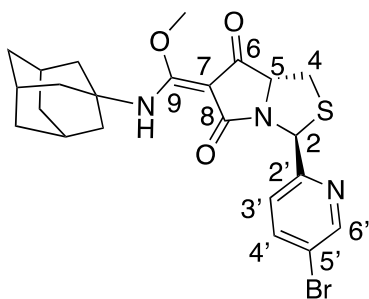
$\text{C}_{21}\text{H}_{20}\text{O}_3\text{N}_2\text{ClS}$ $[\text{M}+\text{H}]^+$ 415.0878 and 417.0848; found 415.0879 and 417.0847.

(2*S*,5*R*)-7-(Adamantylaminomethylene)-1-aza-9-methoxy-6,8-dioxo-2-(pyridin-2-yl)-3-thiabicyclo[3.3.0]octane, 37a



Yield (37 mg, 43%); yellow oil; $R_f = 0.57$ (petrol : EtOAc; 1 : 3); $[\alpha]_D^{25} = -110.0$ ($c = 1.00$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1635 (s, C=O), 1684 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ 9.80 (br. s, 1H, NH), 8.58 (d, $J = 4.7$ Hz, 1H, H6'), 7.66 (app td, $J = 7.7, 1.8$ Hz, 1H, H4'), 7.40 (d, $J = 7.9$ Hz, 1H, H3'), 7.18 (dd, $J = 7.5, 4.9$ Hz, 1H, H5'), 6.49 (s, 1H, H2), 4.57 (app t, $J = 7.7$ Hz, 1H, H5), 4.31 (s, 3H, OCH_3), 3.35 (dd, $J = 10.9, 7.4$ Hz, 1H, H4_B), 3.05 (dd, $J = 10.9, 8.0$ Hz, 1H, H4_A), 2.13 (br. s, 3H, Adamantyl-CH), 2.01 (br. s, 6H, Adamantyl-CH₂), 1.69 (br. s, 6H, Adamantyl-CH₂); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ 193.6 (C6), 176.6 (C8), 169.1 (C9), 160.5 (C2'), 149.8 (C6'), 137.0 (C4'), 122.7 (C3'), 119.9 (C5'), 87.0 (C7), 70.1 (C5), 64.6 (C2), 62.4 (OCH_3), 55.1 (Adamantyl-C), 42.2 (Adamantyl-CH₂), 36.1 (Adamantyl-CH₂), 34.0 (C4), 29.5 (Adamantyl-CH); m/z (ESI⁺) 426 ($[\text{M}+\text{H}]^+$, 100%); HRMS (ESI⁺) m/z calcd for $\text{C}_{23}\text{H}_{28}\text{O}_3\text{N}_3\text{S}$ $[\text{M}+\text{H}]^+$ 426.1846; found 426.1847.

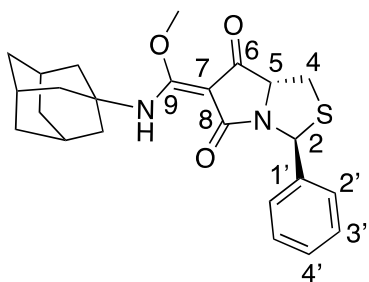
(2*S*,5*R*)-7-(Adamantylaminomethylene)-1-aza-2-(5-bromopyridin-2-yl)-9-methoxy-6,8-dioxo-3-thiabicyclo[3.3.0]octane, 37c



Yield (13 mg, 11%); pale yellow oil; $R_f = 0.32$ (petrol : EtOAc; 7 : 3); $[\alpha]_D^{25} = -132.2$ ($c = 1.0$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1600 (s, C=C), 1636 (s, C=O), 1687 (s, C=O), 3273 (br, N-H); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ 9.78 (br. s, 1H, NH), 8.63 (d, $J = 1.9$ Hz, 1H, H6'), 7.78 (dd, $J = 8.3, 2.3$ Hz, 1H, H4'), 7.32 (d, $J = 8.3$ Hz, 1H, H3'), 6.43 (s, 1H, H2), 4.54 (app t, $J = 7.7$ Hz, 1H, H5), 4.30 (s, 3H, OCH_3), 3.34 (dd, $J = 11.0, 7.4$ Hz, 1H, H4_B), 3.05 (dd, $J = 11.0, 8.0$ Hz, 1H, H4_A), 2.13 (br. s, 3H, Adamantyl-CH), 2.01 (br. s, 6H, Adamantyl-CH₂),

1.69 (br. s, 6H, Adamantyl-CH₂); ¹³C{¹H} NMR (CDCl₃, 126 MHz): δ 192.4 (C6), 174.8 (C8), 169.1 (C9), 159.1 (C2'), 150.8 (C6'), 139.5 (C4'), 121.4 (C3'), 119.6 (C5'), 86.9 (C7), 70.1 (C5), 64.1 (C2), 62.4 (OCH₃), 55.1 (Adamantyl-C), 42.2 (Adamantyl-CH₂), 36.1 (Adamantyl-CH₂), 34.1 (C4), 29.5 (Adamantyl-CH); *m/z* (ESI⁺) 504 and 506 ([M+H]⁺, 100%); HRMS (ESI⁺) *m/z* calcd for C₂₃H₂₇O₃N₃BrS [M+H]⁺ 504.0951 and 506.0931; found 504.0952 and 506.0930.

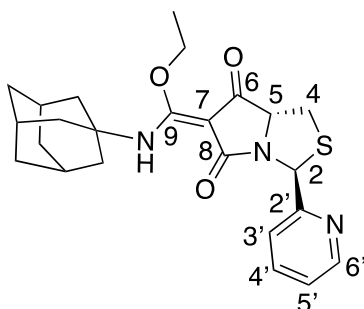
(2*S*,5*R*)-7-(Adamantylaminomethylene)-1-aza-9-methoxy-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]octane, 37e



Yield (22 mg, 31%); pale yellow oil; *R_f* = 0.27 (petrol : EtOAc; 4 : 1); [α]_D²⁵ = -110.3 (*c* = 1.00, CHCl₃); *v*_{max}/cm⁻¹ 1636 (s, C=O), 1682 (s, C=O), 3298 (br. N-H); ¹H NMR (CDCl₃, 400 MHz): δ 9.80 (br. s, 1H, NH), 7.47 (d, *J* = 7.0 Hz, 2H, H₂'),

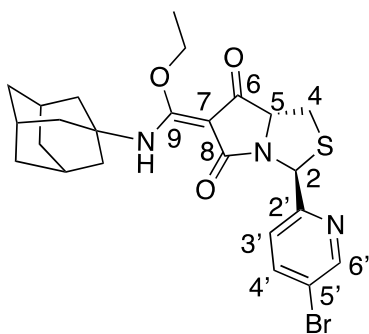
7.34 (t, *J* = 7.4 Hz, 2H, H₃'), 7.26 – 7.28 (m, 1H, H₄'), 6.43 (s, 1H, H₂), 4.42 (app t, *J* = 7.6 Hz, 1H, H₅), 4.31 (s, 3H, OCH₃), 3.28 (dd, *J* = 11.1, 7.4 Hz, 1H, H_{4B}), 3.04 (dd, *J* = 11.1, 8.0 Hz, 1H, H_{4A}), 2.13 (br. s, 3H, Adamantyl-CH), 2.01 (br. s, 6H, Adamantyl-CH₂), 1.69 (br. s, 6H, Adamantyl-CH₂); ¹³C{¹H} NMR (CDCl₃, 126 MHz): δ 192.2 (C6), 174.9 (C8), 169.0 (C9), 141.4 (C1'), 128.7 (C3'), 127.9 (C4'), 126.5 (C2'), 87.1 (C7), 69.6 (C5), 63.9 (C2), 62.4 (OCH₃), 55.1 (Adamantyl-C), 42.2 (Adamantyl-CH₂), 36.1 (Adamantyl-CH₂), 34.1 (C4), 29.5 (Adamantyl-CH); *m/z* (ESI⁺) 425 ([M+H]⁺, 100%); HRMS (ESI⁺) *m/z* calcd for C₂₄H₂₉O₃N₂S [M+H]⁺ 425.1893; found 425.1892.

(2*S*,5*R*)-7-(Adamantylaminomethylene)-1-aza-9-ethoxy-6,8-dioxo-2-(pyridin-2-yl)-3-thiabicyclo[3.3.0]octane, 38a-1



Yield (26 mg, 29%, an inseparable 1 : 0.2 mixture of C-9/C-6 O-alkylated isomers); yellow oil; $R_f = 0.51$ (petrol : EtOAc; 2 : 3); $\nu_{\max}/\text{cm}^{-1}$ 1636 (s, C=O), 1682 (s, C=O), 3298 (br. s, N-H); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): major C-9 O-alkylated isomer: δ 9.89 (br. s, 1H, NH), 8.59 (d, $J = 4.7$ Hz, 1H, H6'), 7.66 (app td, $J = 7.7, 1.8$ Hz, 1H, H4'), 7.40 (d, $J = 7.9$ Hz, 1H, H3'), 7.18 (dd, $J = 8.0, 5.3$ Hz, 1H, H5'), 6.49 (s, 1H, H2), (s, 6.17, H2 minor, C-6 O-alkylated isomer), 4.75 (br. s, 2H, OCH_2CH_3), 4.56 (app t, $J = 7.8$ Hz, 1H, H5), 3.37 – 3.31 (m, 1H, H4_B), 3.04 (dd, $J = 10.9, 8.1$ Hz, 1H, H4_A), 2.13 (br. s, 3H, Adamantyl-CH), 2.04 (br. s, 6H, Adamantyl- CH_2), 1.68 (br. s, 6H, Adamantyl- CH_2), 1.47 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3); $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 126 MHz): major C-9 O-alkylated isomer: δ 196.2 (C6), 180.7 (C8), 167.9 (C9), 160.5 (C2'), 149.8 (C6'), 137.0 (C4'), 122.7 (C3'), 119.9 (C5'), 87.4 (C7), 72.1 (OCH_2CH_3), 70.1 (C5), 64.6 (C2), 54.8 (Adamantyl-C), 42.2 (Adamantyl- CH_2), 36.1 (Adamantyl- CH_2), 34.1 (C4), 29.5 (Adamantyl-CH), 15.5 (OCH_2CH_3); minor C6 O-alkylated isomer (not all peaks shown): δ 196.2 (C6), 177.2 (C8), 162.1 (C9), 96.9 (C7); m/z (ESI⁺) 440 ($[\text{M}+\text{H}]^+$, 100%); HRMS (ESI⁺) m/z calcd for $\text{C}_{24}\text{H}_{30}\text{O}_3\text{N}_3\text{S}$ $[\text{M}+\text{H}]^+$ 440.2008; found 440.2002.

(2*S*,5*R*)-7-(Adamantylaminomethylene)-1-aza-2-(5-bromopyridin-2-yl)-9-ethoxy-6,8-dioxo-3-thiabicyclo[3.3.0]octane, 38c-1



Yield (18 mg, 17%, an inseparable 1 : 0.2 mixture of C-9/C-6

O-alkylated isomers); pale yellow oil; $R_f = 0.33$ (petrol :

EtOAc; 7 : 3); $\nu_{\max}/\text{cm}^{-1}$ 1635 (s, C=O), 1685 (s, C=O); ^1H

NMR (CDCl_3 , 400 MHz): major C-9 O-alkylated isomer: δ

9.87 (br. s, 1H, NH), 8.62 (d, $J = 2.3$ Hz, 1H, H6'), 7.78 (dd, J

= 8.3, 2.3 Hz, 1H, H4'), 7.32 (d, $J = 8.3$ Hz, 1H, H3'), 6.43 (s, 1H, H2), (s, 6.10, H2 minor, C-6

O-alkylated isomer), 4.62 – 4.85 (m, 2H, OCH_2CH_3), 4.53 (app t, $J = 7.7$ Hz, 1H, H5), 3.34

(dd, $J = 11.0, 7.4$ Hz, 1H, H4_B), 3.04 (dd, $J = 11.0, 8.0$ Hz, 1H, H4_A), 2.13 (br. s, 3H,

Adamantyl-CH), 2.04 (br. s, 6H, Adamantyl-CH₂), 1.69 (br. s, 6H, Adamantyl-CH₂), 1.47 (t, J

= 7.1 Hz, 3H, OCH_2CH_3); $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 126 MHz): major C-9 O-alkylated isomer: δ

196.1 (C6), 180.6 (C8), 167.9 (C9), 159.2 (C2'), 150.8 (C6'), 139.5 (C4'), 121.4 (C3'), 119.6

(C5'), 87.3 (C7), 72.1 (OCH_2CH_3), 70.0 (C5), 64.2 (C2), 54.9 (Adamantyl-C), 42.2

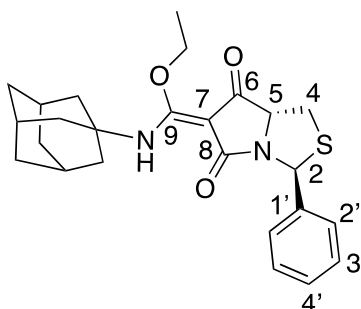
(Adamantyl-CH₂), 36.1 (Adamantyl-CH₂), 34.1 (C4), 29.5 (Adamantyl-CH), 15.5 (OCH_2CH_3);

minor C6 O-alkylated isomer (not all peaks shown): δ 175.0 (C8), 161.0 (C9), 97.0 (C7);

m/z (ESI⁺) 518 and 520 ($[\text{M}+\text{H}]^+$, 100%); HRMS (ESI⁺) m/z calcd for $\text{C}_{24}\text{H}_{29}\text{O}_3\text{N}_3\text{BrS}$ $[\text{M}+\text{H}]^+$

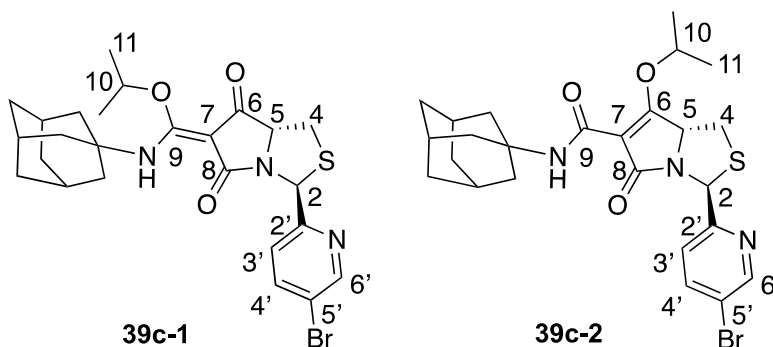
518.11075 and 520.10870; found 518.1107 and 520.1086.

(2*S*,5*R*)-7-(Adamantylaminomethylene)-1-aza-9-ethoxy-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]octane, 38e-1



Yield (24 mg, 32%, an inseparable 1 : 0.1 mixture of C-9/C-6 O-alkylated isomers); pale yellow oil; $R_f = 0.33$ (petrol : EtOAc; 4 : 1); $\nu_{\max}/\text{cm}^{-1}$ 1635 (s, C=O), 1685 (s, C=O); ^1H NMR (CDCl_3 , 400 MHz): major C-9 O-alkylated isomer: δ 9.89 (br. s, 1H, NH), 7.46 (d, $J = 7.1$ Hz, 2H, H_{2'}), 7.34 (t, $J = 7.4$ Hz, 2H, H_{3'}), 7.24 – 7.28 (m, 1H, H_{4'}), 6.43 (s, 1H, H₂), (s, 6.14, H₂ minor, C-6 O-alkylated isomer), 4.69 – 4.79 (m, 2H, OCH_2CH_3), 4.41 (app t, $J = 7.6$ Hz, 1H, H₅), 3.27 (dd, $J = 11.1, 7.4$ Hz, 1H, H_{4B}), 3.03 (dd, $J = 11.1, 7.9$ Hz, 1H, H_{4A}), 2.13 (br. s, 3H, Adamantyl-CH), 2.04 (br. s, 6H, Adamantyl- CH_2), 1.69 (br. s, 6H, Adamantyl- CH_2), 1.46 (t, $J = 7.2$ Hz, 3H, OCH_2CH_3); $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 126 MHz): major C-9 O-alkylated isomer: δ 196.1 (C₆), 181.1 (C₈), 167.9 (C₉), 141.4 (C_{1'}), 128.7 (C_{3'}), 127.9 (C_{4'}), 126.5 (C_{2'}), 87.5 (C₇), 72.0 (OCH_2CH_3), 69.6 (C₅), 63.9 (C₂), 54.8 (Adamantyl-C), 42.2 (Adamantyl- CH_2), 36.1 (Adamantyl- CH_2), 34.1 (C₄), 29.5 (Adamantyl-CH), 15.5 (OCH_2CH_3); minor C₆ O-alkylated isomer (not all peaks shown): δ 175.1 (C₈), 160.5 (C₉), 97.3 (C₇); m/z (ESI⁺) 439 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for $\text{C}_{25}\text{H}_{31}\text{O}_3\text{N}_2\text{S}$ [M+H]⁺ 439.2050; found 439.2048.

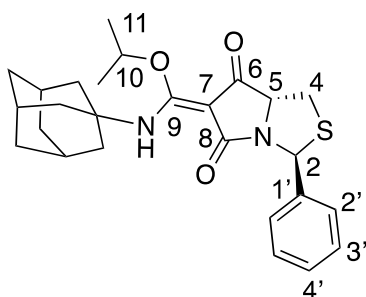
(2*S*,5*R*)-7-(Adamantylaminomethylene)-1-aza-2-(5-bromopyridin-2-yl)-9-isopropoxy-6,8-dioxo-3-thiabicyclo[3.3.0]octane, **39c-1** and (2*S*,5*R*)-7-(Adamantylaminocarbonyl)-1-aza-2-(5-bromopyridin-2-yl)-6-isopropoxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, **39c-2**



Yield (12 mg, 15%, an inseparable 1 : 1 mixture of C9/C6 O-alkylated isomers); pale yellow oil; $R_f = 0.30$ (petrol : EtOAc; 9 : 1); $\nu_{\max}/\text{cm}^{-1}$ 1635 (s, C=O), 1687 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): C-9 O-alkylated isomer **39c-1**: δ 10.09 (br. s, 1H, NH), 8.63 (s, 1H, H6'), 7.79 (app td, $J = 8.1, 2.3$ Hz, 1H, H4'), 7.33 (d, $J = 8.3$ Hz, 1H, H3'), 6.43 (s, 1H, H2), 5.87 (sept, $J = 6.1$ Hz, 1H, H10), 4.53 (app t, $J = 7.6$ Hz, 1H, H5), 3.33 – 3.37 (m, 1H, H4_B), 3.05 (dd, $J = 11.0, 7.8$ Hz, 1H, H4_A), 2.13 (br. s, 3H, Adamantyl-CH), 2.06 (br. s, 6H, Adamantyl-CH₂), 1.68 (br. s, 6H, Adamantyl-CH₂), 1.41 (d, $J = 6.1$ Hz, 3H, H11_B), 1.40 (d, $J = 6.1$ Hz, 3H, H11_A); C6 O-alkylated isomer **39c-2**: δ 8.63 (s, 1H, H6'), 8.10 (br. s, 1H, NH), 7.79 (app td, $J = 8.1, 2.3$ Hz, 1H, H4'), 7.29 (d, $J = 8.3$ Hz, 1H, H3'), 6.23 (s, 1H, H2), 5.63 (sept, $J = 6.1$ Hz, 1H, H10), 4.80 (dd, $J = 8.7, 6.8$ Hz, 1H, H5), 3.29 – 3.33 (m, 1H, H4_B), 2.94 (dd, $J = 10.8, 8.7$ Hz, 1H, H4_A), 2.04 – 2.06 (br. s, 3H, Adamantyl-CH), 2.04 (br. s, 6H, Adamantyl-CH₂), 1.68 (br. s, 6H, Adamantyl-CH₂), 1.36 (d, $J = 6.1$ Hz, 3H, H11_B), 1.32 (d, $J = 6.1$ Hz, 3H, H11_A); $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 126 MHz): C-9 O-alkylated isomer **39c-1**: δ 192.7 (C6, only seen by HMBC), 176.2 (C8), 166.8 (C9), 159.2 (C2'), 150.8 (C6'), 139.5 (C4'), 121.5 (C3'), 119.6 (C5'), 87.6 (C7), 80.3 (C10), 69.9 (C5), 64.4 (C2), 54.7 (Adamantyl-C), 42.2 (Adamantyl-CH₂), 36.6 (Adamantyl-CH₂), 34.2 (C4), 29.6 (Adamantyl-CH), 23.0, 22.8, (C11); C6 O-alkylated isomer

39c-2: δ 176.6 (C6, only seen by HMBC), 174.5 (C8), 160.0 (C9), 158.1 (C2'), 151.0 (C6'), 139.7 (C4'), 121.6 (C3'), 120.0 (C5'), 102.8 (C7), 79.0 (C10), 66.8 (C5), 62.1 (C2), 51.8 (Adamantyl-C), 41.7 (Adamantyl-CH₂), 36.2 (Adamantyl-CH₂), 33.9 (C4), 29.5 (Adamantyl-CH), 22.9, 22.7 (C11); m/z (ESI⁺) 532 and 534 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for C₂₅H₃₁O₃N₃BrS [M+H]⁺ 532.1264 and 534.1244; found 532.1266 and 534.1246.

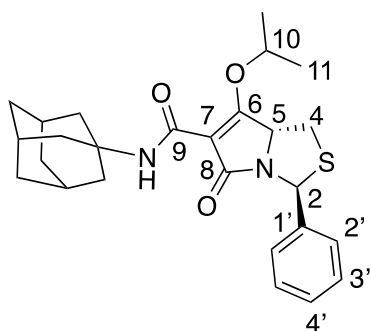
(2S,5R)-7-(Adamantylaminomethylene)-1-aza-9-isopropoxy-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]octane, 39e-1



Yield (24 mg, 27%); pale yellow oil; R_f = 0.33 (petrol : EtOAc; 4 : 1); $[\alpha]_D^{25}$ = -118.5 (c = 1.00, CHCl₃); $\nu_{\max}/\text{cm}^{-1}$ 1633 (s, C=O), 1684 (s, C=O); ¹H NMR (CDCl₃, 400 MHz): δ 10.12 (br. s, 1H, NH), 7.47 (d, J = 7.1 Hz, 2H, H2'), 7.34 (t, J = 7.4 Hz, 2H, H3'), 7.24 – 7.28 (m, 1H, H4'), 6.43 (s, 1H, H2), 5.89 (sept, J = 6.1 Hz, 1H, H10), 4.41 (app t, J = 7.6 Hz, 1H, H5), 3.27 (dd, J = 11.1, 7.4 Hz, 1H, H4_B), 3.03 (dd, J = 11.1, 7.9 Hz, 1H, H4_A), 2.13 (br. s, 3H, Adamantyl-CH), 2.04 (br. s, 6H, Adamantyl-CH₂), 1.69 (br. s, 6H, Adamantyl-CH₂), 1.41 (d, J = 6.1 Hz, 3H, H11_B), 1.40 (d, J = 6.1 Hz, 3H, H11_A); ¹³C{¹H} NMR (CDCl₃, 126 MHz): δ 192.4 (C6), 175.6 (C8), 166.7 (C9), 141.4 (C1'), 128.7 (C3'), 127.9 (C4'), 126.5 (C2'), 87.7 (C7), 80.1 (C10), 69.5 (C5), 64.1 (C2), 54.6 (Adamantyl-C), 42.2 (Adamantyl-CH₂), 36.2 (Adamantyl-CH₂), 34.2 (C4), 29.5 (Adamantyl-CH), 23.0, 22.9 (C11);

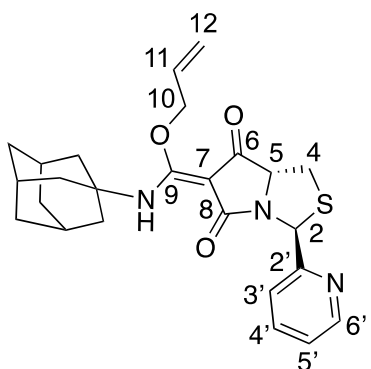
m/z (ESI⁺) 453 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for C₂₆H₃₃O₃N₂S [M+H]⁺ 453.2206; found 453.2208.

(2*S*,5*R*)-7-(Adamantylaminocarbonyl)-1-aza-6-isopropoxy-8-oxo-2-phenyl-3-thiabicyclo[3.3.0]oct-6-ene, 39e-2



Yield (22 mg, 24%); pale yellow oil; $R_f = 0.30$ (petrol : EtOAc; 4 : 1); $[\alpha]_D^{25} = -58.3$ ($c = 1.00$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1654 (s, C=O), 1686 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 500 MHz): δ 8.17 (br. s, 1H, NH), 7.45 (d, $J = 7.6$ Hz, 2H, H2'), 7.35 (t, $J = 7.4$ Hz, 2H, H3'), 7.27 – 7.32 (m, 1H, H4'), 6.23 (s, 1H, H2), 5.67 (sept, $J = 6.1$ Hz, 1H, H10), 4.66 (dd, $J = 8.9$, 6.5 Hz, 1H, H5), 3.25 (dd, $J = 10.9$, 6.5 Hz, 1H, H4_B), 2.90 (dd, $J = 10.9$, 8.9 Hz, 1H, H4_A), 2.06 (br. s, 9H, Adamantyl- CH_2 and Adamantyl- CH), 1.69 (br. s, 6H, Adamantyl- CH_2), 1.39 (d, $J = 6.1$ Hz, 3H, H11_B), 1.35 (d, $J = 6.1$ Hz, 3H, H11_A); $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 126 MHz): δ 175.6 (C6), 174.3 (C8), 160.2 (C9), 140.2 (C1'), 128.9 (C3'), 128.3 (C4'), 126.5 (C2'), 103.1 (C7), 78.9 (C10), 66.6 (C5), 62.0 (C2), 51.8 (Adamantyl-C), 41.7 (Adamantyl- CH_2), 36.6 (Adamantyl- CH_2), 34.0 (C4), 29.6 (Adamantyl- CH), 22.7, 22.6 (C11); m/z (ESI⁺) 453 ($[\text{M}+\text{H}]^+$, 100%); HRMS (ESI⁺) m/z calcd for $\text{C}_{26}\text{H}_{33}\text{O}_3\text{N}_2\text{S}$ $[\text{M}+\text{H}]^+$ 453.2206; found 453.2208.

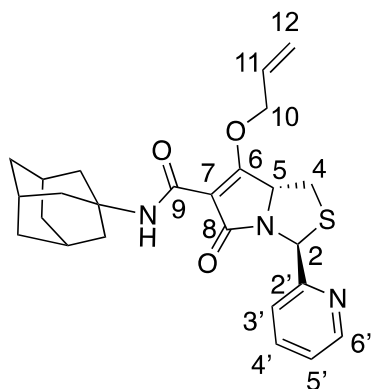
(2*S*,5*R*)-7-(Adamantylaminomethylene)-9-allyloxy-1-aza-6,8-dioxo-2-(pyridin-2-yl)-3-thiabicyclo[3.3.0]octane, 40a-1



Yield (7 mg, 5%, calculated from crude $^1\text{H NMR}$ as the isolated product was contaminated with reduced DEAD); yellow oil; $R_f = 0.65$ (petrol : EtOAc; 1 : 1); $\nu_{\text{max}}/\text{cm}^{-1}$ 1635 (s, C=O), 1719 (s, C=O), 3299 (br. s, N-H); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ 9.94 (br. s, 1H, NH), 8.59 (d, $J = 4.9$ Hz, 1H, H6'), 7.67 (app td, $J = 7.7$, 1.7 Hz, 1H, H4'), 7.40 (d, $J = 7.8$ Hz, 1H, H3'), 7.18 (dd, $J = 7.5$, 4.9 Hz,

1H, H5'), 6.49 (s, 1H, H2), 5.99 – 6.13 (m, 1H, H11), 5.43 (d, $J = 17.1$ Hz, 1H, H12_B), 5.34 (d, $J = 10.3$ Hz, 1H, H12_A), 5.21 (br. s, 2H, H10), 4.57 (app t, $J = 7.7$ Hz, 1H, H5), 3.35 (dd, $J = 10.9, 7.4$ Hz, 1H, H4_B), 3.04 (dd, $J = 10.9, 8.1$ Hz, 1H, H4_A), 2.12 (br. s, 3H, Adamantyl-CH), 2.03 (br. s, 6H, Adamantyl-CH₂), 1.67 (br. s, 6H, Adamantyl-CH₂); ¹³C{¹H} NMR (CDCl₃, 126 MHz): δ 191.2 (C6), 173.1 (C8), 167.6 (C9), 160.4 (C2'), 149.8 (C6'), 137.0 (C4'), 131.8 (C11), 122.7 (C3'), 120.3 (C12), 119.9 (C5'), 87.4 (C7), 76.3 (C10), 70.1 (C5), 64.6 (C2), 55.0 (Adamantyl-C), 42.2 (Adamantyl-CH₂), 36.1 (Adamantyl-CH₂), 34.1 (C4), 29.5 (Adamantyl-CH); m/z (ESI⁺) 452 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for C₂₅H₃₀O₃N₂S [M+H]⁺ 452.2202; found 452.2204.

(2*S*,5*R*)-7-(Adamantylaminocarbonyl)-6-allyloxy-1-aza-8-oxo-2-(pyridin-2-yl)-3-thiabicyclo[3.3.0]oct-6-ene, 40a-2

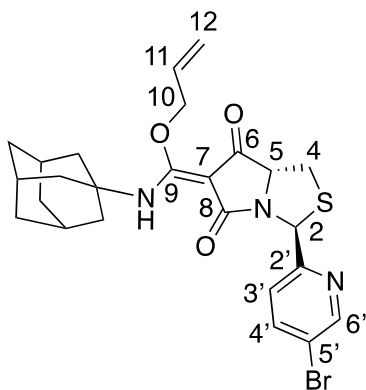


Yield (12 mg, 14%); yellow oil; $R_f = 0.51$ (petrol : EtOAc; 1 : 1); $[\alpha]_D^{25} = -42.4$ ($c = 0.60$, CHCl₃); $\nu_{\max}/\text{cm}^{-1}$ 1655 (s, C=O), 1687 (s, C=O), 3300 (br. s, N-H); ¹H NMR (CDCl₃, 500 MHz): δ 8.59 (d, $J = 4.9$ Hz, 1H, H6'), 8.08 (br. s, 1H, NH), 7.68 (app td, $J = 7.7, 1.8$ Hz, 1H, H4'), 7.37 (d, $J = 7.8$ Hz, 1H, H3'), 7.21 (dd, $J = 7.5, 4.9$ Hz, 1H, H5'), 6.29 (s, 1H, H2), 5.96 – 6.05 (m,

1H, H11), 5.44 (d, $J = 17.2$ Hz, 1H, H12_B), 5.34 (d, $J = 10.5$ Hz, 1H, H12_A), 5.08 – 5.19 (m, 2H, H10), 4.93 (dd, $J = 8.9, 6.8$ Hz, 1H, H5), 3.34 (dd, $J = 10.7, 6.8$ Hz, 1H, H4_B), 2.96 (dd, $J = 10.7, 8.9$ Hz, 1H, H4_A), 2.06 (br. s, 9H, Adamantyl-CH₂ and Adamantyl-CH), 1.67 (br. s, 6H, Adamantyl-CH₂); ¹³C{¹H} NMR (CDCl₃, 126 MHz): δ 176.7 (C6), 173.7 (C8), 159.9 (C9), 159.3 (C2'), 150.0 (C6'), 137.2 (C4'), 131.8 (C11), 123.1 (C3'), 120.1 (C12), 120.0 (C5'), 103.6 (C7), 75.4 (C10), 66.3 (C5), 62.3 (C2), 51.8 (Adamantyl-C), 41.7 (Adamantyl-CH₂),

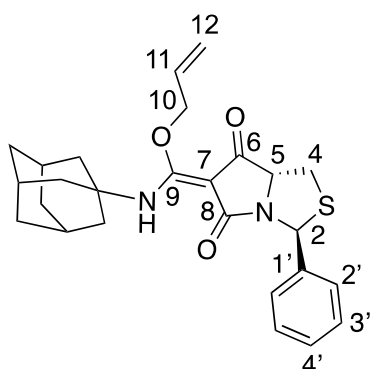
36.6 (Adamantyl-CH₂), 33.6 (C4), 29.6 (Adamantyl-CH); *m/z* (ESI⁺) 452 ([M+H]⁺, 100%); HRMS (ESI⁺) *m/z* calcd for C₂₅H₃₀O₃N₂S [M+H]⁺ 452.2202; found 452.2204.

(2*S*,5*R*)-7-(Adamantylaminomethylene)-9-alkoxy-1-aza-2-(5-bromopyridin-2-yl)-6,8-dioxo-3-thiabicyclo[3.3.0]octane, 40c-1



Yield (10 mg, 11%, isolated as 1 : 0.2 mixture of C-9/C-6 O-alkylated isomers); pale yellow oil; *R_f* = 0.54 (petrol : EtOAc; 7 : 3); *v*_{max}/cm⁻¹ 1636 (s, C=O), 1693 (s, C=O), 3295 (br. s, N-H); ¹H NMR (CDCl₃, 400 MHz): major C-9 O-alkylated isomer: δ 9.92 (br. s, 1H, NH), 8.63 (d, *J* = 2.3 Hz, 1H, H6'), 7.78 (dd, *J* = 8.3, 2.3 Hz, 1H, H4'), 7.32 (d, *J* = 8.3 Hz, 1H, H3'), 6.43 (s, 1H, H2), 6.24 (s, H2 minor, C-6 O-alkylated isomer), 5.99 – 6.15 (m, 1H, H11), 5.43 (d, *J* = 17.1 Hz, 1H, H12_B), 5.35 (d, *J* = 10.4 Hz, 1H, H12_A), 5.19 (br. s, 2H, H10), 4.71 (m, H5 minor), 4.53 (app t, *J* = 7.5 Hz, 1H, H5), 3.34 (dd, *J* = 11.0, 7.4 Hz, 1H, H4_B), 3.04 (dd, *J* = 11.0, 8.0 Hz, 1H, H4_A), 2.12 (br. s, 3H, Adamantyl-CH), 2.03 (br. s, 6H, Adamantyl-CH₂), 1.68 (br. s, 6H, Adamantyl-CH₂); ¹³C{¹H} NMR (CDCl₃, 126 MHz): major C-9 O-alkylated isomer: δ 192.5 (C6), 173.2 (C8), 167.6 (C9), 159.1 (C2'), 150.8 (C6'), 139.5 (C4'), 131.7 (C11), 121.4 (C3'), 120.4 (C5'), 119.6 (C12), 87.4 (C7), 76.3 (C10), 70.0 (C5), 64.2 (C2), 55.0 (Adamantyl-C), 42.2 (Adamantyl-CH₂), 36.1 (Adamantyl-CH₂), 34.1 (C4), 29.5 (Adamantyl-CH); minor C-6 O-alkylated isomer (not all peaks shown): δ 175.0 (C6), 169.5 (C8), 159.6 (C9), 100.2 (C7); *m/z* (ESI⁺) 530 and 532 ([M+H]⁺, 100%); HRMS (ESI⁺) *m/z* calcd for C₂₅H₂₉O₃N₃BrS [M+H]⁺ 530.1108 and 532.1087; found 530.1109 and 532.1087.

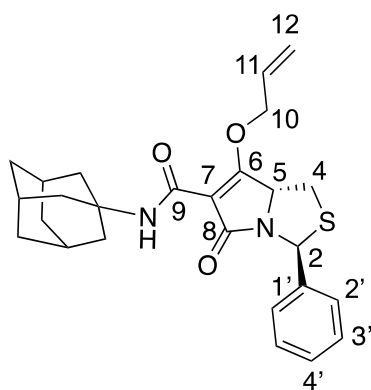
(2*S*,5*R*)-7-(Adamantylaminomethylene)-9-allyloxy-1-aza-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]octane, 40e-1



Yield (25 mg, 21%); yellow oil; $R_f = 0.32$ (petrol : EtOAc; 4 : 1); $[\alpha]_D^{25} = -110.5$ ($c = 1.00$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1635 (s, C=O), 1685 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 500 MHz): δ 9.95 (br. s, 1H, NH), 7.47 (d, $J = 7.5$ Hz, 2H, H2'), 7.34 (t, $J = 7.6$ Hz, 2H, H3'), 7.25 – 7.28 (m, 1H, H4', obscured by solvent), 6.43 (s, 1H,

H2), 6.01 – 6.12 (m, 1H, H11), 5.43 (d, $J = 17.1$ Hz, 1H, H12_B), 5.34 (d, $J = 10.3$ Hz, 1H, H12_A), 5.20 (br. s, 2H, H10), 4.41 (app t, $J = 7.6$ Hz, 1H, H5), 3.27 (dd, $J = 11.1, 7.4$ Hz, 1H, H4_B), 3.03 (dd, $J = 11.1, 8.0$ Hz, 1H, H4_A), 2.12 (br. s, 3H, Adamantyl-CH), 2.02 (br. s, 6H, Adamantyl-CH₂), 1.68 (br. s, 6H, Adamantyl-CH₂); $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 126 MHz): δ 192.4 (C6), 175.1 (C8), 167.6 (C9), 141.3 (C1'), 131.8 (C11), 128.7 (C3'), 127.9 (C4'), 126.5 (C2'), 120.3 (C12), 87.6 (C7), 76.2 (C10), 69.5 (C5), 63.9 (C2), 54.9 (Adamantyl-C), 42.2 (Adamantyl-CH₂), 36.1 (Adamantyl-CH₂), 34.1 (C4), 29.5 (Adamantyl-CH); m/z (ESI⁺) 451 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for $\text{C}_{26}\text{H}_{31}\text{O}_3\text{N}_2\text{S}$ [M+H]⁺ 451.2050; found 451.2049.

(2*S*,5*R*)-7-(Adamantylaminocarbonyl)-6-allyloxy-1-aza-8-oxo-2-phenyl-3-thiabicyclo[3.3.0]oct-6-ene, 40e-2

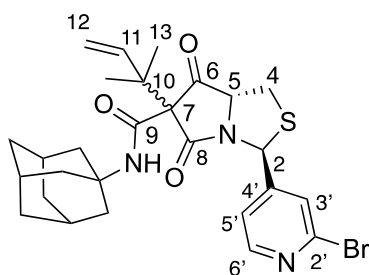


Yield (46 mg, 38%); pale yellow oil; $R_f = 0.16$ (petrol : EtOAc; 4 : 1); $[\alpha]_D^{25} = -50.2$ ($c = 1.00$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1656 (s, C=O), 1686 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ 8.10 (br. s, 1H, NH), 7.44 (d, $J = 7.2$ Hz, 2H, H2'), 7.29 – 7.37 (m, 3H, H4' and H3'), 6.21 (s, 1H, H2), 5.92 – 6.06 (m, 1H, H11), 5.43 (d,

$J = 17.2$ Hz, 1H, H12_B), 5.33 (d, $J = 10.4$ Hz, 1H, H12_A), 5.13 (d, $J = 5.7$ Hz, 2H, H10), 4.74 (dd,

$J = 9.1, 6.4$ Hz, 1H, H₅), 3.26 (dd, $J = 10.9, 6.4$ Hz, 1H, H_{4B}), 2.92 (dd, $J = 10.9, 9.1$ Hz, 1H, H_{4A}), 2.05 (br. s, 6H, Adamantyl-CH₂), 1.96 (br. s, 3H, Adamantyl-CH), 1.67 (br. s, 6H, Adamantyl-CH₂); ¹³C NMR (CDCl₃, 101 MHz): δ 176.0 (C6), 173.7 (C8), 159.9 (C9), 140.0 (C1'), 131.8 (C11), 128.8 (C3'), 128.4 (C4'), 126.5 (C2'), 119.9 (C12), 103.8 (C7), 75.4 (C10), 66.1 (C5), 61.7 (C2), 51.8 (Adamantyl-C), 41.6 (Adamantyl-CH₂), 36.5 (Adamantyl-CH₂), 33.8 (C4), 29.5 (Adamantyl-CH); m/z (ESI⁺) 451 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for C₂₆H₃₁O₃N₂S [M+H]⁺ 451.2050; found 451.2049.

(2*S*,5*R*)-7-(Adamantylaminocarbonyl)-2-(2-bromopyridin-4-yl)-1-aza-7-(2-methylbut-3-en-2-yl)-6,8-dioxo--3-thiabicyclo[3.3.0]octane, 41b

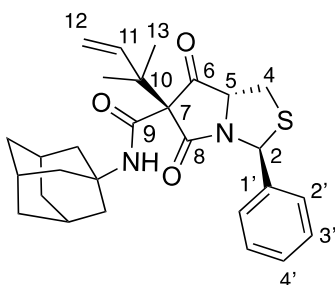


Synthesised from **30b** (200 mg, 0.4 mmol); yield (23 mg, 10%, an inseparable 1 : 1 mixture of 7*R*/*S* diastereomers); yellow oil; $R_f = 0.21$ (petrol : EtOAc; 9 : 1); ¹H NMR (CDCl₃, 400 MHz): δ 8.38 (d, $J = 5.1$ Hz, 1H, H_{6'}), 8.36 (d, $J = 5.5$ Hz,

1H, H_{6'}), 7.51 (s, 1H, H_{3'}), 7.43 (s, 1H, H_{3'}), 7.22 (d, $J = 5.1$ Hz, 1H, H_{5'}, another set of H_{5'} of the other diastereomer obscured by solvent peak), 6.96 (br. s, 2H, NH), 6.47 (s, 1H, H₂, 7*R*), 6.45 (s, 1H, H₂, 7*S*), 6.19 (br. s, 1H, H₁₁), 6.00 (dd, $J = 17.4, 10.7$ Hz, 1H, H₁₁), 5.09 – 5.28 (m, 4H, H₁₂), 4.17 – 4.27 (m, 2H, H₅), 3.32 (dd, $J = 10.8, 6.8$ Hz, 1H, H_{4B}, 7*S*), 3.23 – 3.29 (m, 1H, H_{4B}, 7*R*), 3.03 – 3.11 (m, 1H, H_{4A}, 7*S*), 2.87 (dd, $J = 13.6, 7.7$ Hz, 1H, H_{4A}, 7*R*), 2.08 (br. s, 6H, Adamantyl-CH), 1.99 (br. s, 12H, Adamantyl-CH₂), 1.67 (br. s, 12H, Adamantyl-CH₂), 1.36 (s, 3H, H_{13B}, 7*S*), 1.31 (s, 3H, H_{13A}, 7*S*), 1.29 (s, 3H, H_{13B}, 7*R*), 1.25 (s, 3H, H_{13A}, 7*R*); ¹³C{¹H} NMR (CDCl₃, 126 MHz): **7*R*-41b**: δ 201.4 (C6), 172.1 (C8), 163.1 (C9), 151.6 (C6'), 150.7 (C4'), 142.1 (C2'), 139.4 (C11), 125.0 (C3'), 120.4 (C5'), 116.1 (C12), 69.9 (C7), 64.4 (C5), 60.5 (C2), 53.1 (Adamantyl-C), 44.7 (C10), 41.3 (Adamantyl-CH₂), 36.3

(Adamantyl-CH₂), 33.8 (C₄), 29.5 (Adamantyl-CH), 26.3 (C_{13B}), 25.9 (C_{13A}); **7S-41b**: δ 199.6 (C₆), 172.2 (C₈), 160.1 (C₉), 151.3 (C_{6'}), 150.8 (C_{4'}), 143.0 (C_{2'}), 139.4 (C₁₁), 125.4 (C_{3'}), 120.5 (C_{5'}), 115.4 (C₁₂), 70.0 (C₇), 66.2 (C₅), 61.1 (C₂), 53.1 (Adamantyl-C), 44.7 (C₁₀), 41.4 (Adamantyl-CH₂), 36.4 (Adamantyl-CH₂), 34.2 (C₄), 29.5 (Adamantyl-CH), 23.5 (C_{13B}), 23.0 (C_{13A}); m/z (ESI⁺) 558 and 560 ([M+H]⁺, 100%), C₂₇H₃₃O₃N₃BrS ([M+H]⁺).

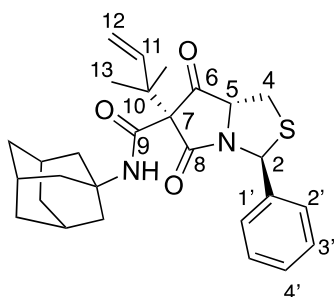
(2S,5R,7S)-7-(Adamantylaminocarbonyl)-1-aza-7-(2-methylbut-3-en-2-yl)-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]octane, 7S-41e



Synthesised from **30e** (330 mg, 0.7 mmol); yield (49 mg, 15%); pale yellow oil; R_f = 0.34 (petrol : EtOAc; 9 : 1); $[\alpha]_D^{25}$ = -115.8 (c = 1.00, CHCl₃); $\nu_{\max}/\text{cm}^{-1}$ 1661 (s, C=O), 1697 (s, C=O), 1770 (s, C=O); ¹H NMR (CDCl₃, 400 MHz): δ 7.38 – 7.42 (m, 2H, H_{2'}), 7.34 – 7.38 (m, 2H, H_{3'}), 7.31 – 7.35 (m, 1H, H_{4'}), 7.11 (br. s, 1H, NH), 6.55 (s, 1H, H₂), 6.00 (dd, J = 17.3, 10.8 Hz, 1H, H₁₁), 5.07 – 5.15 (m, 2H, H₁₂), 4.33 (dd, J = 9.9, 6.7 Hz, 1H, H₅), 3.30 (dd, J = 10.8, 6.7 Hz, 1H, H_{4B}), 2.99 – 3.07 (m, 1H, H_{4A}), 2.06 (br. s, 3H, Adamantyl-CH), 1.99 (br. s, 6H, Adamantyl-CH₂), 1.66 (br. s, 6H, Adamantyl-CH₂), 1.26 (s, 3H, H_{13A}), 1.32 (s, 3H, H_{13B}); ¹³C NMR (CDCl₃, 101 MHz): δ 200.1 (C₆), 171.7 (C₈), 160.3 (C₉), 142.1 (C₁₁), 139.5 (C_{1'}), 129.0 (C_{3'}), 128.7 (C_{4'}), 126.4 (C_{2'}), 115.5 (C₁₂), 70.1 (C₅), 69.1 (C₇), 62.9 (C₂), 52.9 (Adamantyl-C), 44.6 (C₁₀), 41.3 (Adamantyl-CH₂), 36.4 (Adamantyl-CH₂), 34.0 (C₄), 29.5 (Adamantyl-CH), 23.4 (C_{13B}), 23.0 (C_{13A}); m/z (ESI⁺) 479

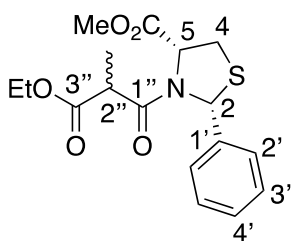
([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for C₂₈H₃₅O₃N₂S [M+H]⁺ 479.2363; found 479.2365.

(2*S*,5*R*,7*R*)-7-(Adamantylaminocarbonyl)-1-aza-7-(2-methylbut-3-en-2-yl)-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]octane, 7*R*-41e



Synthesised from **30e** (330 mg, 0.7 mmol); yield (3%, calculated from crude ^1H NMR, characterisation based a 1 : 0.8 mixture of 7*R*/*S* diastereomers of 8 mg isolated from column chromatography); pale yellow oil; R_f = 0.59 (petrol : EtOAc; 9 : 1); $\nu_{\text{max}}/\text{cm}^{-1}$ 1661 (s, C=O), 1698 (s, C=O), 1772 (s, C=O); ^1H NMR (CDCl_3 , 500 MHz): δ (NH not observed), 7.27 – 7.43 (m, 5H, H4', H3' and H2'), 6.58 (s, 1H, H2), 5.99 (dd, J = 17.3, 10.7 Hz, 1H, H11), 5.04 – 5.17 (m, 2H, H12), 4.62 (dd, J = 8.9, 7.6 Hz, 1H, H5), 3.27 (m, 1H, H4_B), 2.90 (dd, J = 10.6, 8.9 Hz, 1H, H4_A), 2.05 (br. s, 3H, Adamantyl-CH), 1.93 (br. s, 6H, Adamantyl-CH₂), 1.65 (br. s, 6H, Adamantyl-CH₂), 1.25 (s, 3H, H13_B), 1.24 (s, 3H, H13_A); $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 126 MHz): δ 201.8 (C6), 170.9 (C8), 162.9 (C9), 142.2 (C11), 139.3 (C1'), 129.0 (C3'), 128.6 (C4'), 126.2 (C2'), 116.9 (C12), 69.5 (C5), 68.0 (C7), 62.3 (C2), 53.0 (Adamantyl-C), 44.6 (C10), 41.3 (Adamantyl-CH₂), 36.3 (Adamantyl-CH₂), 32.9 (C4), 29.5 (Adamantyl-CH), 26.0 (C13); m/z (ESI⁺) 479 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for C₂₈H₃₅O₃N₂S [M+H]⁺ 479.2363; found 479.2365.

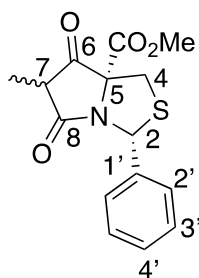
(2*R*,5*R*)-1-(3-Ethoxy-2-methyl-3-oxopropanoyl)-5-methoxycarbonyl-2-phenyl-1,3-thiazolidine, 43e



Synthesised from **24e** (1 g, 3 mmol) by procedure **G**; yield (195 mg, 19%); yellow oil; inseparable 0.8: 1 C2'' *R*/*S* diastereomers; R_f = 0.18 (petrol : EtOAc; 4 : 1); ^1H NMR (CDCl_3 , 400 MHz): δ 7.62 (d, J = 7.0 Hz, 2H, H2' major), 7.24 – 7.40 (m, 8H, H4' and H3' major and minor and H2' minor, obscured by solvent peak), 6.30 (s, 1H, H2 major), 6.08 (s, 1H,

H2 minor), 5.16 – 5.33 (m, 1H, H5 minor), 4.85 – 5.04 (m, 1H, H5 major), 4.01 – 4.32 (m, 4H, OCH₂CH₃), 3.77 (s, 3H, CO₂CH₃ major), 3.72 (s, 3H, CO₂CH₃ minor), 3.26 – 3.40 (m, 3H, H2'' and H4), 3.10 – 3.26 (m, 3H, H2'' and H4), 1.31 – 1.42 (m, 3H, OCH₂CH₃), 1.16 – 1.28 (m, 3H, OCH₂CH₃), 1.04 – 1.19 (d, *J* = 6.9 Hz, 3H, CH₃ major), 0.99 – 1.06 (m, 3H, CH₃ minor); *m/z* (ESI⁺) 374 ([M+Na]⁺, 100%); HRMS (ESI⁺) *m/z* calcd for C₁₇H₂₁O₅N₂NaS [M+Na]⁺ 374.1033; found 374.1033.

(2*R*,5*R*)-1-Aza-6,8-dioxo-7-methyl-5-methoxycarbonyl-2-phenyl-3-thiabicyclo[3.3.0]octane, 44e



Synthesised from **43e** (890 mg, 2.5 mmol) by general procedure **C**; yield

(272 mg, 35%, an inseparable 0.3 : 1 mixture of diastereomers, 7*R*/*S*);

yellow oil; *R_f* = 0.25 (EtOAc: MeOH; 8 : 1); ¹H NMR (CDCl₃, 400 MHz): δ

7.50 (d, *J* = 7.2 Hz, 2H, H2'), 7.26 – 7.37 (m, 3H, H4' and H3'), 6.61 (s, H2

minor), 6.57 (s, 1H, H2 major), 3.99 (d, *J* = 11.1 Hz, H4_B minor), 3.92 (d, *J* = 11.5 Hz, 1H,

H4_B major), 3.65 (s, 3H, CO₂CH₃ major), 3.55 (d, *J* = 7.4 Hz, 1H, H7), 3.52 (s, CO₂CH₃ minor),

3.16 (d, *J* = 11.5 Hz, 1H, H4_A major), 3.09 – 3.13 (m, H4_A minor), 1.51 (d, *J* = 7.8 Hz, CH₃

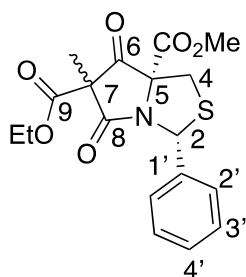
minor), 1.33 (d, *J* = 7.4 Hz, 3H, CH₃ major); ¹³C NMR (CDCl₃, 101 MHz): δ 201.4 (C6), 171.6

(C8), 166.8 (CO₂CH₃), 137.9 (C1'), 128.7, 128.7, 127.3 (C2', C3' and C4'), 82.7 (C5), 64.2 (C2

major), 63.7 (C2, minor), 53.9 (CO₂CH₃), 48.3 (C7), 35.7 (C4), 8.5 (CH₃); *m/z* (ESI⁺) 306

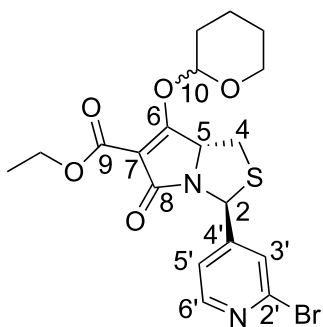
([M+H]⁺, 100%); HRMS (ESI⁺) *m/z* calcd for C₁₅H₁₆O₄NS [M+H]⁺ 306.0795; found 306.0797.

(2*R*,5*R*)-1-Aza-7-ethoxycarbonyl-7-methyl-5-methoxycarbonyl-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]octane, 45e



Synthesised from **44e** (180 mg, 0.6 mmol) by general procedure **H**; yield (66 mg, 29%, an inseparable 1 : 0.5 mixture of diastereomers, *C7R/S*); pale yellow oil; $R_f = 0.25$ (petrol : EtOAc; 85 : 15); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ 7.57 (d, $J = 7.2$ Hz, 2H, H_2' major), 7.52 (d, $J = 7.2$ Hz, 2H, H_2' minor), 7.26 – 7.40 (m, 6H, H_4' and H_3' major and minor), 6.60 (s, 1H, H_2 major), 6.19 (s, 1H, H_2 minor), 4.32 (q, $J = 7.1$ Hz, 2H, OCH_2CH_3 minor), 4.20 (q, $J = 7.1$ Hz, 2H, OCH_2CH_3 major), 3.85 (d, $J = 11.5$ Hz, 1H, $\text{H}_{4\text{B}}$ major), 3.76 (d, $J = 11.5$ Hz, 1H, $\text{H}_{4\text{B}}$ minor), 3.69 (s, 3H, CO_2CH_3 minor), 3.59 (s, 3H, CO_2CH_3 major), 3.16 (d, $J = 11.5$ Hz, 1H, $\text{H}_{4\text{A}}$ major), 3.15 (d, $J = 11.5$ Hz, 1H, $\text{H}_{4\text{A}}$ minor), 1.79 (s, 3H, CH_3 minor), 1.59 (s, 3H, CH_3 major), 1.38 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3 minor), 1.24 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3 major); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ 196.8 (C6), 172.6 (C8), 169.3, 168.2, 165.8, 164.6 (CO_2CH_3 and C9 major and minor), 138.6, 137.5 ($\text{C}1'$ major and minor), 128.6, 128.5, 128.5, 128.3, 127.3, 127.0 ($\text{C}2'$, $\text{C}3'$ and $\text{C}4'$ major and minor), 82.6 (C5 major), 78.9 (C5 minor), 66.4 (C7), 64.7, 63.3 (C2 major and minor), 62.5, 62.1 (OCH_2CH_3 major and minor), 53.7, 53.5 (CO_2CH_3 major and minor), 37.8, 37.0 (C4 major and minor), 16.6, 14.1 (OCH_2CH_3 major and minor), 7.9 (CH_3); m/z (ESI^+) 378 ($[\text{M}+\text{H}]^+$, 100%); HRMS (ESI^+) m/z calcd for $\text{C}_{18}\text{H}_{20}\text{O}_6\text{NS}$ $[\text{M}+\text{H}]^+$ 378.1006; found 378.1013.

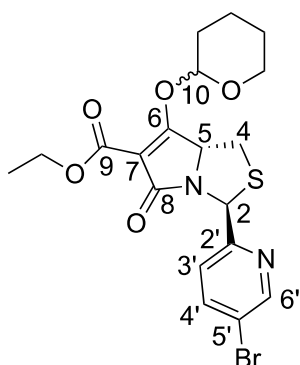
(2*S*,5*R*)-1-Aza-2-(2-bromopyridin-4-yl)-7-ethoxycarbonyl-8-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-3-thiabicyclo[3.3.0]oct-6-ene, 47b



Synthesised from **25b** (100 mg, 0.3 mmol) by general procedure I; yield (70 mg, 57%); pale yellow oil; inseparable 1 : 1 10*R/S* diastereomers; $R_f = 0.34$ (petrol : EtOAc; 4 : 1); $\nu_{\max}/\text{cm}^{-1}$ 1709 (s, C=O), 1747 (s, C=O), 1780 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ 8.34 (d, $J = 5.2$ Hz, 1H, H6'), 8.33 (d, $J = 5.2$

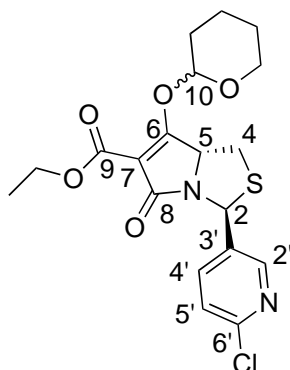
Hz, 1H, H6'), 7.56 (s, 1H, H3'), 7.50 (s, 1H, H3'), 7.15 – 7.21 (m, 2H, H5'), 6.50 (s, 1H, H2), 6.44 (s, 1H, H2), 4.50 (dd, $J = 10.2, 6.7$ Hz, 1H, H10), 4.45 (dd, $J = 10.0, 6.8$ Hz, 1H, H10), 4.00 – 4.31 (m, 6H, OCH_2CH_3 and H5), 3.65 – 3.89 (m, 2H, Tetrahydropyranyl OCH_2), 3.33 – 3.57 (m, 2H, Tetrahydropyranyl OCH_2), 3.27 – 3.33 (m, 2H, H4_B), 3.06 – 3.19 (m, 2H, H4_A), 1.88 – 1.99 (m, 4H, Tetrahydropyranyl CH_2), 1.58 – 1.82 (m, 4H, Tetrahydropyranyl CH_2), 1.52 (br. s, 4H, Tetrahydropyranyl CH_2), 1.21 – 1.26 (m, 6H, OCH_2CH_3); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ 200.8, 200.3 (C6), 167.6, 166.7 (C8), 163.3, 163.2 (C9), 151.8, 151.7 (C6'), 150.7, 150.6 (C4'), 143.4, 143.1 (C2'), 124.5, 124.4 (C3'), 120.3 (C5'), 80.5, 79.3 (C7), 70.6, 70.3 (C10), 69.8, 69.7 (Tetrahydropyranyl OCH_2), 67.2, 67.1 (C5), 62.7, 62.7 (C2), 59.3, 59.1 (OCH_2CH_3), 34.0, 33.9 (C4), 26.2, 25.8, 25.7, 25.6, 25.2 (Tetrahydropyranyl CH_2), 22.9, 22.9 (Tetrahydropyranyl CH_2), 14.0, 13.9 (OCH_2CH_3); m/z (APCI⁺) 469 and 471 ($[\text{M}+\text{H}]^+$, 100%); HRMS (APCI⁺) m/z calcd for $\text{C}_{19}\text{H}_{22}\text{O}_5\text{N}_2\text{BrS}$ $[\text{M}+\text{H}]^+$ 469.0427 and 471.0407; found 469.0428 and 471.0405.

(2*S*,5*R*)-1-Aza-2-(5-bromopyridin-2-yl)-7-ethoxycarbonyl-8-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-3-thiabicyclo[3.3.0]oct-6-ene, 47c



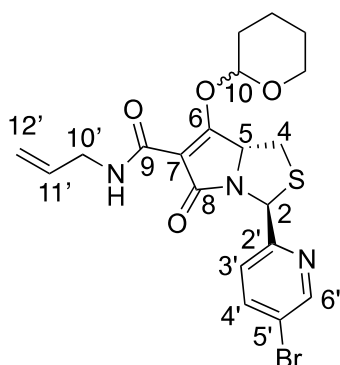
Synthesised from **25c** (100 mg, 0.3 mmol) by general procedure I; yield (72 mg, 59%); yellow oil; inseparable 1 : 1 10*R/S* diastereomers; $R_f = 0.42$ (petrol : EtOAc; 4 : 1); $\nu_{\max}/\text{cm}^{-1}$ 1707 (s, C=O), 1748 (s, C=O), 1779 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ 8.65 (d, $J = 2.3$ Hz, 1H, H6'), 8.64 (d, $J = 2.2$ Hz, 1H, H6'), 7.82 (dd, $J = 8.3, 2.3$ Hz, 1H, H4'), 7.79 (dd, $J = 8.3, 2.3$ Hz, 1H, H4'), 7.28 (d, $J = 8.3$ Hz, 1H, H3'), 7.24 (d, 1H, H3', obscured by solvent peak), 6.58 (s, 1H, H2), 6.52 (s, 1H, H2), 4.69 (dd, $J = 9.9, 6.8$ Hz, 1H, H10), 4.64 (dd, $J = 9.9, 6.8$ Hz, 1H, H10), 4.07 – 4.32 (m, 6H, OCH_2CH_3 and H5), 3.90 – 3.99 (m, 2H, Tetrahydropyranyl OCH_2), 3.41 – 3.54 (m, 2H, Tetrahydropyranyl OCH_2), 3.34 (dd, $J = 6.8, 2.1$ Hz, 1H, H4_B), 3.31 (dd, $J = 6.8, 2.1$ Hz, 1H, H4_A), 3.18 (t, $J = 10.1$ Hz, 1H, H4_B), 3.11 (t, $J = 10.1$ Hz, 1H, H4_A), 1.99 (br. s, 4H, Tetrahydropyranyl CH_2), 1.77 – 1.96 (m, 4H, Tetrahydropyranyl CH_2), 1.46 – 1.60 (m, 4H, Tetrahydropyranyl CH_2), 1.22 – 1.27 (m, 6H, OCH_2CH_3); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ 201.7, 201.2 (C6), 167.8, 167.2 (C8), 163.5, 163.4 (C9), 157.6, 157.5 (C2'), 151.3, 151.3 (C6'), 139.8, 139.8 (C4'), 120.3, 120.2 (C3'), 120.0 (C5'), 80.4, 79.3 (C7), 71.4, 71.2 (C10), 70.1, 69.9 (Tetrahydropyranyl OCH_2), 69.7, 69.6 (C5), 62.7, 62.7 (C2), 62.2, 62.1 (OCH_2CH_3), 33.8, 33.7 (C4), 26.2, 25.9 (Tetrahydropyranyl CH_2), 25.7, 25.2 (Tetrahydropyranyl CH_2), 23.1, 22.9 (Tetrahydropyranyl CH_2), 14.0, 14.0 (OCH_2CH_3); m/z (APCI⁺) 469 and 471 ($[\text{M}+\text{H}]^+$, 100%); HRMS (APCI⁺) m/z calcd for $\text{C}_{19}\text{H}_{22}\text{O}_5\text{N}_2\text{BrS}$ $[\text{M}+\text{H}]^+$ 469.0427 and 471.0407; found 469.0420 and 471.0399.

(2*S*,5*R*)-1-Aza-2-(6-chloropyridin-3-yl)-7-ethoxycarbonyl-8-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-3-thiabicyclo[3.3.0]oct-6-ene, 47d



Synthesised from **25d** (80 mg, 0.2 mmol) by general procedure I; yield (53 mg, 53%); greenish yellow oil; inseparable 1 : 1 10*R/S* diastereomers; $R_f = 0.31$ (petrol : EtOAc; 4 : 1); $\nu_{\max}/\text{cm}^{-1}$ 1711 (s, C=O), 1749 (s, C=O), 1779 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ 8.45 (s, 1H, H2'), 8.42 (s, 1H, H2'), 7.62 – 7.70 (m, 2H, H4'), 7.29 – 7.35 (m, 2H, H5'), 6.58 (s, 1H, H2), 6.52 (s, 1H, H2), 4.50 – 4.58 (m, 1H, H10), 4.47 (dd, $J = 9.7, 6.9$ Hz, 1H, H10), 4.11 – 4.28 (m, 6H, OCH_2CH_3 and H5), 3.80 – 3.97 (m, 2H, Tetrahydropyranyl OCH_2), 3.43 – 3.52 (m, 2H, Tetrahydropyranyl OCH_2), 3.36 – 3.43 (m, 1H, H4_B), 3.28 – 3.36 (m, 1H, H4_B), 3.16 – 3.23 (m, 1H, H4_A), 3.08 – 3.16 (m, 1H, H4_A), 1.75 – 2.03 (m, 4H, Tetrahydropyranyl CH_2), 1.58 – 1.75 (m, 4H, Tetrahydropyranyl CH_2), 1.45 – 1.58 (m, 4H, Tetrahydropyranyl CH_2), 1.24 (t, $J = 7.1$ Hz, 6H, OCH_2CH_3); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ 201.2, 200.7 (C6), 167.8, 167.3 (C8), 163.4, 163.3 (C9), 151.5 (C2'), 147.6, 147.5 (C6'), 137.1, 137.1 (C4'), 134.7, 134.5 (C3'), 124.4 (C5'), 80.3, 79.2 (C7), 70.9, 70.8 (C10), 70.3, 70.1 (Tetrahydropyranyl OCH_2), 67.1 (C5), 62.8, 62.7 (C2), 59.2, 59.1 (OCH_2CH_3), 33.9, 33.8 (C4), 26.2 (Tetrahydropyranyl CH_2), 25.2 (Tetrahydropyranyl CH_2), 23.0, 22.9 (Tetrahydropyranyl CH_2), 13.9 (OCH_2CH_3); m/z (ESI⁺) 425 ([M+H]⁺, 100%); and 427 ([M+H]⁺, 33%); HRMS (APCI⁺) m/z calcd for $\text{C}_{19}\text{H}_{22}\text{O}_5\text{N}_2\text{ClS}$ [M+H]⁺ 425.0933 and 427.0903; found 425.0937 and 427.0907.

(2*S*,5*R*)-1-Aza-7-(allylaminocarbonyl)-2-(5-bromopyridin-2-yl)-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 48c

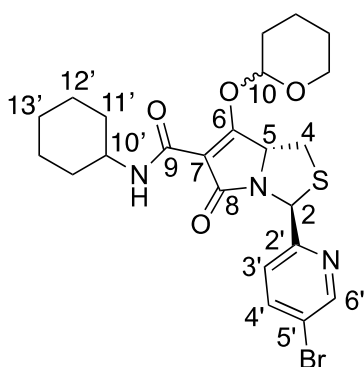


Synthesised from **47c** (50 mg, 0.1 mmol) by general procedure **D** with allylamine; yield (42 mg, 83%); yellow oil; separable 2 : 1 diastereomers, 10*R*/*S*; $R_f = 0.81$ (minor) and 0.69 (major) (petrol : EtOAc; 1 : 4); major isomer: ^1H NMR (CDCl_3 , 400 MHz): δ (*NH* not observed), 8.63 (d, $J = 2.8$ Hz, 1H,

$\text{H6}'$), 7.85 (dd, $J = 8.3, 2.3$ Hz, 1H, $\text{H5}'$), 7.15 (d, $J = 8.4$ Hz, 1H, $\text{H3}'$), 6.25 (s, 1H, H2), 5.76 – 5.90 (m, 1H, $\text{H11}'$), 5.35 (dd, $J = 4.4, 3.1$ Hz, 1H, H10), 5.27 (d, $J = 17.2$ Hz, 1H, $\text{H12}'_{\text{B}}$), 5.11 (d, $J = 10.4$ Hz, 1H, $\text{H12}'_{\text{A}}$), 3.86 – 4.05 (m, 5H, Tetrahydropyranyl OCH_2 , H5 and $\text{H10}'$), 3.39 – 3.46 (m, 1H, H4_{B}), 3.27 (d, $J = 10.2$ Hz, 1H, H4_{A}), 1.65 – 1.82 (br. s, 2H, Tetrahydropyranyl CH_2), 1.44 – 1.59 (m, 2H, Tetrahydropyranyl CH_2), 1.06 – 1.08 (td, $J = 7.1, 2.6$ Hz, 2H, Tetrahydropyranyl CH_2); $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 126 MHz): δ 168.9 (C6), 167.1 (C8), 165.9 (C9), 159.1 (C2'), 151.0 (C6'), 139.9 (C4'), 134.3 (C11'), 121.1 (C3'), 120.1 (C5'), 115.4 (C12'), 78.4 (C7), 69.2 (C10), 66.0 (Tetrahydropyranyl OCH_2), 65.9 (C5), 61.5 (C2), 42.0 (C10'), 31.8 (C4), 31.2 (Tetrahydropyranyl CH_2), 25.6 (Tetrahydropyranyl CH_2), 22.6 (Tetrahydropyranyl CH_2); minor isomer: ^1H NMR (CDCl_3 , 400 MHz): δ (*NH* not observed), 8.40 (d, $J = 2.2$ Hz, 1H, $\text{H6}'$), 7.73 (dd, $J = 8.3, 2.3$ Hz, 1H, $\text{H5}'$), 7.08 (d, $J = 8.3$ Hz, 1H, $\text{H3}'$), 6.13 (s, 1H, H2), 5.77 – 5.92 (m, 1H, H11), 5.32 (d, $J = 6.6$ Hz, 1H, H10), 5.25 (d, $J = 17.2$ Hz, 1H, $\text{H12}'_{\text{B}}$), 5.15 (d, $J = 10.3$ Hz, 1H, $\text{H12}'_{\text{A}}$), 3.82 – 4.17 (m, 5H, Tetrahydropyranyl OCH_2 , H5 and $\text{H10}'$), 3.58 (app d, $J = 11.8$ Hz, 1H, H4_{B}), 3.53 (app d, $J = 10.1$ Hz, 1H, H4_{A}), 1.65 – 1.82 (br. s, 2H, Tetrahydropyranyl CH_2), 1.50 (br. s, 2H, Tetrahydropyranyl CH_2), 1.27 – 1.31 (td, $J = 7.1, 2.6$ Hz, 2H, Tetrahydropyranyl CH_2); $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 126 MHz): δ 169.4 (C6), 165.9 (C8), 165.7 (C9), 158.9 (C2'), 150.0 (C6'), 139.4 (C4'), 133.8 (C11'), 121.1

(C3'), 119.4 (C5'), 116.6 (C12'), 78.4 (C7), 69.1 (C10), 66.7 (Tetrahydropyranyl OCH₂), 65.4 (C5), 61.9 (C2), 42.7 (C10'), 34.3 (C4), 29.7 (Tetrahydropyranyl CH₂), 26.0 (Tetrahydropyranyl CH₂), 22.9 (Tetrahydropyranyl CH₂); *m/z* (ESI⁺) 502 and 504 ([M+Na]⁺, 100%), C₂₀H₂₂O₄N₃BrSNa [M+Na]⁺.

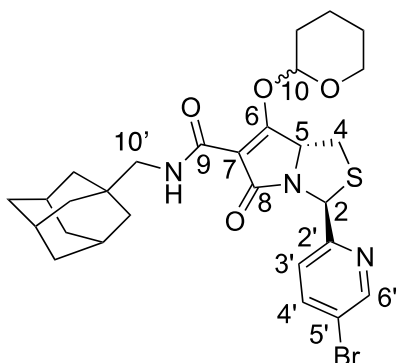
(2*S*,5*R*)-1-Aza-2-(5-bromopyridin-2-yl)-7-(cyclohexylaminocarbonyl)-8-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-3-thiabicyclo[3.3.0]oct-6-ene, 49c



Synthesised from **47c** (50 mg, 0.1 mmol) by general procedure **D** with cyclohexylamine; yield (23 mg, 44%); yellow oil; *R_f* = 0.45 (100% EtOAc); ¹H NMR (CDCl₃, 400 MHz): major isomer isolated from column chromatography (the crude appeared as a 1.5 : 1 mixture of isomers with

another H₂ singlet at 6.13): δ 8.60 (d, *J* = 2.0 Hz, 1H, H_{6'}), 8.10 (br. s, 1H, NH), 7.84 (td, *J* = 8.3, 2.4 Hz, 1H, H_{4'}), 7.12 (d, *J* = 8.2 Hz, 1H, H_{3'}), 6.29 (s, 1H, H₂), 5.21 (d, *J* = 6.2 Hz, 1H, H₁₀), 4.56 (br. s, 1H, H₅), 3.65 – 3.94 (m, 3H, Tetrahydropyranyl OCH₂ and H_{10'}), 3.30 – 3.58 (m, 1H, H_{4_B}), 3.05 – 3.27 (m, 1H, H_{4_A}), 1.88 (br. s), 1.70 (br. s), 1.60 (br. s), 1.53 (br. s), 1.36 (br. s), 1.23 – 1.31 (m), 1.19 (br. s) (3 x Tetrahydropyranyl CH₂, H_{11'} 4H, H_{12'} 4H and H_{13'} 2H, a total of 16H); *m/z* (ESI⁺) 522 and 524 ([M+H]⁺, 100%), C₂₃H₂₉O₄N₃BrS [M+H]⁺.

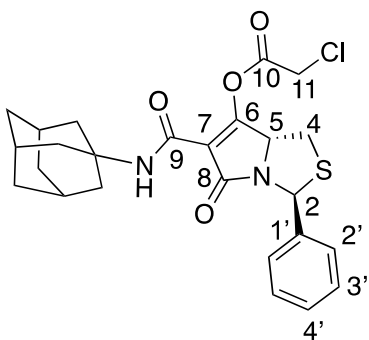
(2*S*,5*R*)-7-(Adamantylmethylaminocarbonyl)-1-aza-2-(5-bromopyridin-2-yl)-8-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-3-thiabicyclo[3.3.0]oct-6-ene, 50c



Synthesised from **47c** (50 mg, 0.1 mmol) by general procedure **D** with 1-adamantylmethylamine; yield (20 mg, 26%, an inseparable mixture of diastereomers, major, minor, C10*R/S* and rotamers, 1 : 0.8 : 0.6 based on H2 chemical shifts); yellow oil; R_f = 0.43 (petrol:

EtOAc; 1 : 1); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ (NH not observed), 8.62 (d, J = 2.3 Hz, 1H, H6'), 7.85 (td, J = 8.3, 2.3 Hz, 1H, H4'), 7.14 (d, J = 8.4 Hz, 1H, H3'), 6.28 (s, 1H, H2 major), 6.14 (s, H2 minor), 6.02 (s, H2 minor), 5.27 (dd, J = 4.8, 2.4 Hz, 1H, H10), 4.15 – 4.26 (m, 1H, H5), 3.78 – 4.03 (m, 2H, Tetrahydropyranyl OCH_2), 3.36 – 3.43 (m, 2H, H10'), 2.96 – 3.27 (m, 2H, H4_A and H4_B), 1.96 (br. s, 9H, Adamantyl- CH_2 and Adamantyl- CH), 1.69 (br. s, 6H, Adamantyl- CH_2), 1.64 (br. s, 2H, Tetrahydropyranyl CH_2), 1.49 (br. s, 2H, Tetrahydropyranyl CH_2), 1.27 – 1.33 (m, 2H, Tetrahydropyranyl CH_2); m/z (ESI⁺) 588 and 590 ($[\text{M}+\text{H}]^+$, 100%), $\text{C}_{28}\text{H}_{35}\text{O}_4\text{N}_3\text{BrS}$ $[\text{M}+\text{H}]^+$.

(2*S*,5*R*)-7-(Adamantylaminocarbonyl)-1-aza-6-chloroacetate-8-oxo-2-phenyl-3-thiabicyclo[3.3.0]oct-6-ene, 54e

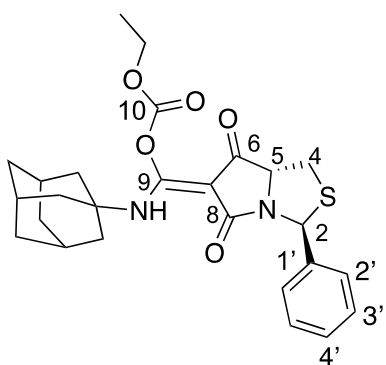


Synthesised from **30e** (90 mg, 0.2 mmol) by procedure **J**; yield (20 mg, 19%); orange oil; R_f = 0.39 (petrol : EtOAc; 85 : 15); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ 7.59 (br. s, 1H, NH), 7.48 (d, J = 7.3 Hz, 2H, H2'), 7.37 (t, J = 7.3 Hz, 2H, H3'), 7.33 (d, J = 7.1 Hz, 1H, H4'), 6.22 (s, 1H, H2), 5.01 (dd, J = 8.6, 6.6 Hz,

1H, H5), 3.93 (s, 2H, H11), 3.32 (dd, J = 11.1, 6.6 Hz, 1H, H4_B), 2.98 (dd, J = 11.1, 8.6 Hz, 1H,

H_{4A}), 2.17 (br. s, 6H, Adamantyl-CH₂), 2.02 (br. s, 3H, Adamantyl-CH), 1.68 (br. s, 6H, Adamantyl-CH₂); ¹³C{¹H} NMR (CDCl₃, 126 MHz): δ 169.4 (C8), 168.0 (C6), 164.8 (C10), 160.3 (C9), 139.1 (C1'), 129.0 (C3'), 128.7 (C4'), 126.7 (C2'), 104.2 (C7), 88.8 (C11), 64.6 (C5), 61.5 (C2), 55.4 (Adamantyl-C), 41.4 (Adamantyl-CH₂), 36.3 (Adamantyl-CH₂), 32.4 (C4), 29.5 (Adamantyl-CH); C₂₅H₂₇O₄N₂ClS.

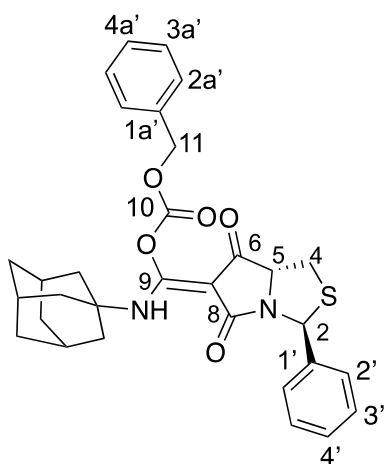
(2*S*,5*R*)-7-(Adamantylaminomethylene)-1-aza-9-ethylcarbonate-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]octane, 55e



Synthesised from **30e** (110 mg, 0.3 mmol) by procedure **H**; yield (40 mg, 31%); yellow oil; *R*_f = 0.30 (petrol : EtOAc; 85 : 15); ¹H NMR (CDCl₃, 400 MHz): δ 9.90 (br. s, 1H, NH), 7.46 (d, *J* = 8.1 Hz, 2H, H₂'), 7.33 (t, *J* = 7.4 Hz, 2H, H₃'), 7.26 – 7.28 (m, 1H, H₄', obscured by solvent peak), 6.43 (s,

1H, H₂), 4.75 (app d, *J* = 6.8 Hz, 2H, OCH₂CH₃), 4.40 (app t, *J* = 7.6 Hz, 1H, H₅), 3.27 (dd, *J* = 11.1, 7.4 Hz, 1H, H_{4B}), 3.03 (dd, *J* = 11.1, 7.9 Hz, 1H, H_{4A}), 2.13 (br. s, 3H, Adamantyl-CH), 2.04 (br. s, 6H, Adamantyl-CH₂), 1.69 (br. s, 6H, Adamantyl-CH₂), 1.46 (t, *J* = 7.1 Hz, 3H, OCH₂CH₃); ¹³C{¹H} NMR (CDCl₃, 126 MHz): δ 192.4 (C6), 175.1 (C8), 167.8 (C9), 163.8 (C10), 141.3 (C1'), 128.6 (C3'), 127.8 (C4'), 126.4 (C2'), 87.5 (C7), 72.0 (OCH₂CH₃), 69.5 (C5), 63.9 (C2), 54.7 (Adamantyl-C), 42.1 (Adamantyl-CH₂), 36.1 (Adamantyl-CH₂), 34.0 (C4), 29.4 (Adamantyl-CH), 15.4 (OCH₂CH₃); C₂₆H₃₀O₅N₂S.

(2*S*,5*R*)-7-(Adamantylaminomethylene)-1-aza-9-benzylcarbonate-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]octane, 56e



Synthesised from **55e** (30 mg, 0.1 mmol) by procedure **K**; yield (5 mg, 13%); pale yellow oil; $R_f = 0.32$ (petrol : EtOAc; 85 : 15); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ 9.93 (br. s, 1H, NH), 7.43 – 7.53 (m, 4H, H2' and H2a'), 7.29 – 7.43 (m, 6H, H4', H4a', H3' and H3a'), 6.47 (s, 1H, H2), 5.70 (br. s, 2H, H11), 4.46 (app t, $J = 7.6$ Hz, 1H, H5), 3.31 (dd, $J = 11.1, 7.4$ Hz, 1H, H4_B), 3.07 (dd, $J = 11.1, 7.9$ Hz, 1H, H4_A), 1.99 (br. s, 3H, Adamantyl-CH), 1.89 (br. s, 6H, Adamantyl-CH₂), 1.54 (br. s, 6H, Adamantyl-CH₂); $\text{C}_{31}\text{H}_{32}\text{O}_5\text{N}_2\text{S}$.

6.4 Experimental for Chapter 4

6.4.1 General procedure (L) for synthesis of L-cystine diesters 61-67

To a stirred suspension of L-cystine (1.0 eqv., 1 g, 4.2 mmol) and *p*-toluenesulphonic acid monohydrate (2.5 eqv.) in cyclohexane ($c = 0.2$) was added the respective alcohol (5 eqv.). The mixture was heated under reflux (80 °C) with continuous removal of water using Dean-Stark apparatus for 5 - 16 h. The reaction mixture was then cooled to room temperature and the precipitate was collected by vacuum filtration, washed with DCM and dried to give the corresponding esters of L-cystine as *p*-toluenesulphonate salts **61-67**.

6.4.2 General procedure (M) for synthesis of L-cysteine esters 68-74

A round bottom flask charged with respective L-cystine diester *p*-toluenesulphonate salt (1 eqv., 7.5 mmol) in DCM ($c = 0.15$) was refilled with N_2 for several times. Et_3N (2.5 eqv.) was added followed by dithiothreitol (DTT, 2.5 eqv.) and the mixture was stirred for 24 h.

The reaction mixture was concentrated *in vacuo*; the resulting residue was dissolved in EtOAc and sat. aq. NaHCO₃. The combined organic layer was acidified and extracted with 0.5 M aq. HCl. The combined aqueous layer was basified with powdered NaHCO₃ and re-extracted with EtOAc. The resulting organic layer was dried with MgSO₄, filtered and concentrated *in vacuo*. The crude was further purified with flash chromatography (EtOAc/MeOH) to produce esters of L-cysteine **68-74**.

6.4.3 General procedure (N) for C-acylation of N-acylthiazolidines

Dry pyridine (1.5 eqv.) was added dropwise to a cooled suspension of *N*-acylthiazolidine (1.0 eqv.) and magnesium bromide MgBr₂ (1.5 eqv.) in anhydrous DCM (*c* = 0.15) at 0 °C. The suspension was stirred at this temperature for 30 min and a solution of methoxy(methyl)carbamic chloride **89** (1.5 eqv.) in anhydrous DCM (5 ml) was added dropwise. The reaction mixture was stirred at room temperature for 24 h and then acidified with 2M aq. HCl (or 5% citric acid if pyridyl rings were present) at 0 °C. The aqueous phase extracted with EtOAc, dried with MgSO₄, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography to give the tricarbonyl thiazolidines **92-99**.

Methoxy(methyl)carbamic chloride **89** was synthesised according to literature published procedures.¹⁸⁹ Dry pyridine (8.2 ml, 6 eqv.) was added dropwise to a suspension of triphosgene (5 g, 1 eqv.) and *N,O*-dimethylhydroxylamine hydrochloride (5 g, 3 eqv.) in dry DCM (50 ml) at -78 °C. The mixture was allowed to cool to room temperature and stirred over night for 16 h. The reaction mixture was diluted with distilled water and extracted with diethyl ether. The organic layer was washed sequentially with sat. aq.

NaHCO₃ and brine, dried with anhydrous MgSO₄, filtered and concentrated *in vacuo* (water bath temperature at 25 °C to avoid evaporation of the product) to give carbamic chloride **89** as a yellow oil (4.9 g, 89%). The crude oil was used directly for procedure **N** without further purifications. ¹H NMR (CDCl₃, 400 MHz): δ 3.76 (s, 3H, NOCH₃), 3.32 (s, 3H, NCH₃); ¹³C NMR (CDCl₃, 101 MHz): δ 127.2 (CO), 61.8 (NOCH₃), 36.3 (NCH₃).

6.4.4 General procedure (O) for synthesis of tetramate Weinreb amides 101-109

1,8-Diazabicycloundec-7-ene (DBU, 1.1 eqv.) was added to a solution of tricarbonyl thiazolidine (1.0 eqv.) in anhydrous THF and stirred at room temperature for 24 h. The reaction mixture was then cooled to 0 °C and diluted with distilled water. The mixture was partitioned between diethyl ether (Et₂O) and water. The aqueous layer was then acidified at 0 °C with 2M aq. HCl or 5% citric acid if pyridyl rings were present, extracted with EtOAc, dried with MgSO₄, filtered and concentrated *in vacuo* (water bath at 25 °C) to give the Weinreb amides **101-109**. Unless otherwise stated, the crude from extraction was used for subsequent reactions without further column purification.

6.4.5 General procedure (P) for synthesis of tetramate ketone analogues 112-144

To a vigorously stirring mixture of magnesium turnings (1.1 eqv.) in dry THF (*c* = 0.2), the respective bromide (1 eqv.) and 2 drops of 1,2-bromoethane were added dropwise with gentle reflux. The mixture was heated at 45 °C for 1 h, until all Mg turnings were dissolved and cooled to room temperature. Pyridyl MgBr was prepared with ethyl bromide (0.2 eqv.) with an excess of Mg turnings (2 eqv.). *iPr*Mg-thiazole was prepared by an exchange reaction between *iPr*₂Mg (1.1 eqv.) and 2-bromo-thiazole (1 eqv.) in dry THF at room temperature for 2.5 h. The diisopropylmagnesium salt *iPr*₂Mg was prepared fresh

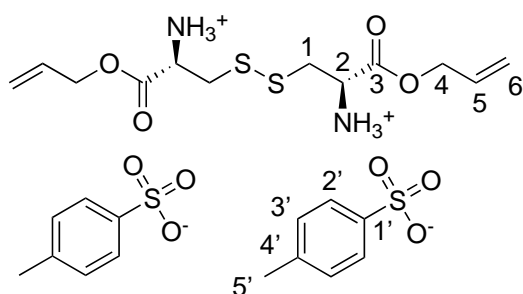
according to a protocol published in literature by adding dry 1,4-dioxane (1.3 eqv.) to a solution of *iPr*MgBr (1 eqv.) in dry THF.^{194,195} The supernatant after stirring for 24 h was used directly for the exchange reaction.

The prepared Grignard reagent (2.4 eqv.) was added to the Weinreb amide (1 eqv.) in anhydrous THF (*c* = 0.05) dropwise at -15 °C. The reaction mixture was stirred at -15 °C for 1 h or 18 h for styrenyl MgBr and pyridyl MgBr and then quenched with sat. aq. NH₄Cl solution at -15 °C. The mixture was acidified with 2M aq. HCl or 5% citric acid if pyridyl rings were present and extracted with EtOAc. The combined organic layer was dried with MgSO₄, filtered and concentrated *in vacuo*. The residue was then purified with flash column chromatography (petroleum ether/EtOAc). The product obtained after column chromatography was dissolved in DCM and washed with 0.5 M aq. HCl or 5% citric acid if pyridyl rings were present to give tetramate ketones **112-144**.

6.4.6 Suzuki-Miyaura cross coupling for synthesis of 160-162 (Procedure Q)

Tetramate analogue (1 eqv.), the respective boronic acid (3 eqv.), Pd(OAc)₂ (0.05 eqv.)/Xphos (0.15 eqv.) (or RuPhos-Pd-G2, 0.15 eqv.) and Na₂CO₃ (6.3 eqv.) were suspended in 1,2-dimethoxyethane (6.3 ml/mmol) in a sealed flask and degassed with N₂. The mixture was refluxed for 18 – 48 h (85 °C). The crude mixture was diluted with EtOAc and filtered through a Celite plug. The filtrate was concentrated *in vacuo* and purified with column chromatography. The product was redissolved in DCM and washed with 0.5 M HCl or 5% citric acid if pyridyl rings were present to wash off chelated metal. The combined organic fractions were dried with MgSO₄, filtered and concentrated *in vacuo* to give the product.

L-cystine allyl ester *p*-toluenesulphonate, 61



Quantitative yield (2.8 g); pale brown solid, m.p.

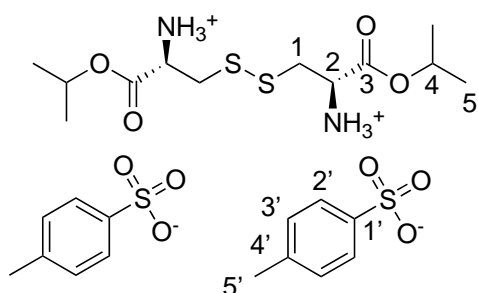
136 °C; $[\alpha]_D^{25} = -35.9$ ($c = 1.00$, MeOH); $\nu_{\max}/\text{cm}^{-1}$

¹ (neat) 2981 (br. s, N-H), 1749 (s, C=O); ¹H NMR

(methanol-*d*₄, 400 MHz): δ 7.71 (d, $J = 8.2$ Hz,

4H, H2'), 7.24 (d, $J = 7.9$ Hz, 4H, H3'), 5.91 – 6.06 (m, 2H, H5), 5.41 (dd, $J = 17.2, 1.4$ Hz, 2H, H6_B), 5.30 (dd, $J = 10.4, 1.2$ Hz, 2H, H6_A), 4.72 – 4.79 (m, 4H, H4), 4.45 – 4.51 (m, 2H, H2), 3.36 (dd, $J = 11.8, 6.0$ Hz, 2H, H1_B), 3.27 – 3.34 (m, 2H, H1_A, obscured by solvent peak), 2.37 (s, 6H, H5'); ¹³C NMR (methanol-*d*₄, 101 MHz): δ 168.7 (C3), 143.4 (C1'), 141.8 (C4'), 132.4 (C5), 129.9 (C2'), 127.0 (C3'), 120.1 (C6), 68.5 (C4), 53.0 (C2), 37.9 (C1), 21.3 (C5'); m/z (ESI⁺) 321 ([M+H]⁺, 100%, M as free base); HRMS (ESI⁺) m/z calcd for C₁₂H₂₁O₄N₂S₂ [M+H]⁺ 321.0937; found 321.0939.

L-cystine isopropyl ester *p*-toluenesulphonate, 62



Yield (87% to quantitative, 2.5 g); white solid, m.p.

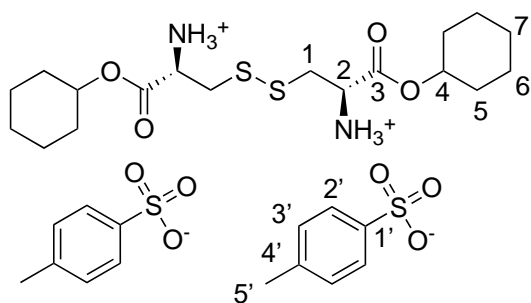
190 °C; $[\alpha]_D^{25} = -47.8$ ($c = 0.86$, MeOH); $\nu_{\max}/\text{cm}^{-1}$

(neat) 2981 (br. s, N-H), 1742 (s, C=O); ¹H NMR

(methanol-*d*₄, 400 MHz): δ 7.72 (d, $J = 8.2$ Hz, 4H,

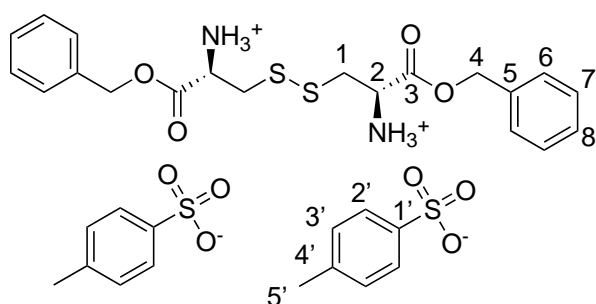
H2'), 7.25 (d, $J = 7.9$ Hz, 4H, H3'), 5.13 (sept, $J = 6.3$ Hz, 2H, H4), 4.39 (dd, $J = 6.9$ Hz, 2H, 5.2 Hz, H2), 3.39 (dd, $J = 14.9, 5.2$ Hz, 2H, H1_B), 3.27 – 3.34 (m, 2H, H1_A, obscured by solvent peak), 2.38 (s, 6H, H5'), 1.33 (s, 6H, H5_B), 1.32 (s, 6H, H5_A); ¹³C NMR (methanol-*d*₄, 101 MHz): δ 168.4 (C3), 143.4 (C1'), 141.8 (C4'), 129.8 (C2'), 127.0 (C3'), 72.8 (C4), 53.2 (C2), 38.2 (C1), 21.9 (C5_B), 21.8 (C5_A), 21.3 (C5'); m/z (ESI⁺) 325 ([M+H]⁺, 100%, M as free base); HRMS (ESI⁺) m/z calcd for C₁₂H₂₅O₄N₂S₂ [M+H]⁺ 325.1250; found 325.1249.

L-cystine cyclohexyl ester *p*-toluenesulphonate, 63



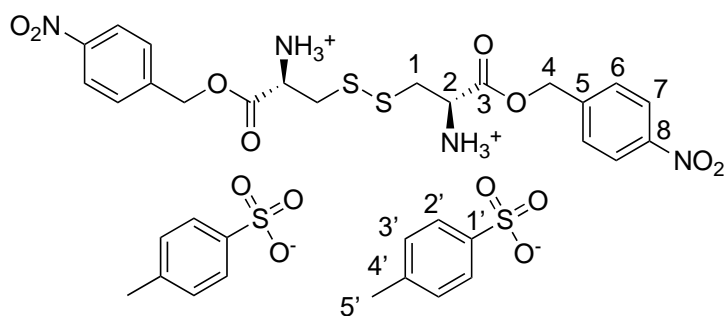
Quantitative yield (3.2 g); white solid, m.p. 196 °C; $[\alpha]_D^{25} = -28.9$ ($c = 1.1$, MeOH); $\nu_{\max}/\text{cm}^{-1}$ (neat) 2933 (br. s, N-H), 1745 (s, C=O); $^1\text{H NMR}$ (methanol- d_4 , 400 MHz): δ 7.71 (d, $J = 8.2$ Hz, 4H, H2'), 7.24 (d, $J = 7.9$ Hz, 4H, H3'), 4.87 – 4.95 (m, 2H, H4), 4.41 (app t, $J = 6.0$ Hz, 2H, H2), 3.35 (dd, $J = 9.6, 6.1$ Hz, 2H, H1_B), 3.29 – 3.32 (m, 2H, H1_A, obscured by solvent peak), 2.37 (s, 6H, H5'), 1.90 (br. s, 4H, H5), 1.76 (br. s, 4H, H5), 1.47 – 1.63 (m, 6H, H6, H7), 1.25 – 1.48 (m, 6H, H6, H7); $^{13}\text{C NMR}$ (methanol- d_4 , 101 MHz): δ 168.4 (C3), 143.4 (C1'), 141.8 (C4'), 129.8 (C2'), 127.0 (C3'), 77.3 (C4), 53.2 (C2), 38.3 (C1), 32.3 (C5), 32.3 (C5), 26.2 (C7), 24.6 (C6), 24.5 (C6), 21.3 (C5'); m/z (ESI⁺) 405 ([M+H]⁺, 100%, M as free base); HRMS (ESI⁺) m/z calcd for C₁₈H₃₃O₄N₂S₂ [M+H]⁺ 405.1876; found 405.1877.

L-cystine benzyl ester *p*-toluenesulphonate, 64



Quantitative yield (3.5 g); off white solid, m.p. 178 °C; $[\alpha]_D^{25} = -25.0$ ($c = 1.3$, MeOH); $\nu_{\max}/\text{cm}^{-1}$ (neat) 2923 (br. s, N-H), 1748 (s, C=O); $^1\text{H NMR}$ (methanol- d_4 , 400 MHz): δ 7.75 (d, $J = 8.2$ Hz, 4H, H2'), 7.40 – 7.44 (m, 4H, H6), 7.33 – 7.40 (m, 6H, H7, H8), 7.24 (d, $J = 7.9$ Hz, 4H, H3'), 5.27 (s, 4H, H4), 4.47 (app t, $J = 5.9$ Hz, 2H, H2), 3.26 – 3.33 (m, 4H, H1, obscured by solvent peak), 2.37 (s, 6H, H5'); $^{13}\text{C NMR}$ (methanol- d_4 , 101 MHz): δ 168.8 (C3), 143.2 (C1'), 141.8 (C4'), 136.0 (C5), 129.8 (C2'), 129.8 (C7), 129.7 (C8), 129.7 (C6), 126.9 (C3'), 69.5 (C4), 53.0 (C2), 37.8 (C1), 21.3 (C5'); m/z (ESI⁺) 421 ([M+H]⁺, 100%, M as free base); HRMS (ESI⁺) m/z calcd for C₂₀H₂₅O₄N₂S₂ [M+H]⁺ 421.1250; found 421.1247.

L-cystine *p*-nitrobenzyl ester *p*-toluenesulphonate, 65



Quantitative yield (4.3 g);

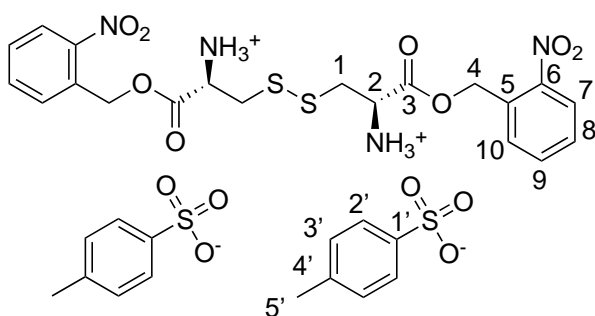
yellow solid, m.p. 209 °C; $[\alpha]_D^{25}$

= -7.43 ($c = 1.1$, MeOH);

$\nu_{\max}/\text{cm}^{-1}$ (neat) 2981 (br. s, N-

H), 1751 (s, C=O); $^1\text{H NMR}$ (methanol- d_4 , 400 MHz): δ 8.17 – 8.25 (m, 4H, H7), 7.70 (d, $J = 8.2$ Hz, 4H, H2'), 7.65 (d, $J = 8.9$ Hz, 2H, H6), 7.59 (d, $J = 8.9$ Hz, 2H, H6), 7.23 (d, $J = 7.9$ Hz, 4H, H3'), 5.35 – 5.45 (m, 4H, H4), 4.57 (dd, $J = 6.6, 5.5$ Hz, 2H, H2), 3.36 – 3.42 (m, 4H, H1, obscured by solvent peak), 2.36 (s, 6H, H5'); $^{13}\text{C NMR}$ (methanol- d_4 , 101 MHz): δ 168.8 (C3), 150.9 (C8), 143.4 (C1'), 141.8 (C4'), 130.1 (C5), 129.8 (C2'), 128.2 (C7), 127.0 (C3'), 124.7 (C6), 124.4 (C6), 64.0 (C4), 53.0 (C2), 38.0 (C1), 21.3 (C5'); m/z (ESI $^+$) 511 ([M+H] $^+$, 100%, M as free base); HRMS (ESI $^+$) m/z calcd for $\text{C}_{20}\text{H}_{23}\text{O}_8\text{N}_4\text{S}_2$ [M+H] $^+$ 511.0952; found 511.0950.

L-cystine *o*-nitrobenzyl ester *p*-toluenesulphonate, 66



Quantitative yield (4.2 g); yellow solid,

m.p. 199 °C; $[\alpha]_D^{25} = -14.1$ ($c = 0.95$,

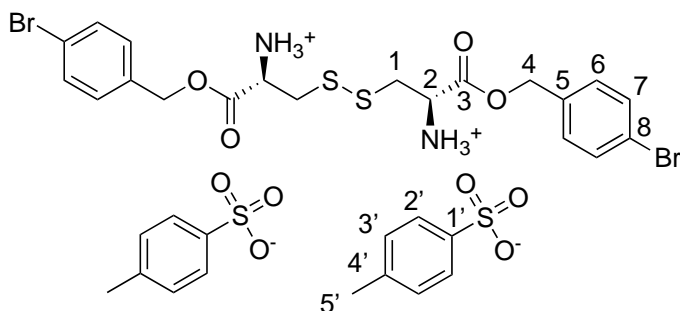
MeOH); $\nu_{\max}/\text{cm}^{-1}$ (neat) 2981 (br. s, N-H),

1753 (s, C=O); $^1\text{H NMR}$ (methanol- d_4 , 400

MHz): δ 8.15 (d, $J = 8.0$ Hz, 2H, H7), 7.80 – 7.72 (m, 8H, H2', H9 and H8), 7.68 – 7.62 (m, 2H, H10), 7.26 (d, $J = 9.0$ Hz, 4H, H3'), 5.67 (s, 4H, H4), 4.60 (dd, $J = 6.9, 5.2$ Hz, 2H, H2), 3.45 (dd, $J = 15.0, 5.2$ Hz, 2H, H1_B), 3.39 (m, 2H, H1_A, obscured by solvent peak), 2.41 (s, 6H, H5'); $^{13}\text{C NMR}$ (methanol- d_4 , 101 MHz): δ 168.7 (C3), 149.3 (C6), 143.4 (C1'), 141.8 (C4'), 135.1 (C10), 131.5 (C5), 131.2 (C9), 130.9 (C8), 129.8 (C2'), 126.9 (C3'), 126.1 (C7),

66.3 (C4), 53.0 (C2), 38.0 (C1), 21.3 (C5'); m/z (ESI⁺) 511 ([M+H]⁺, 100%, M as free base); HRMS (ESI⁺) m/z calcd for C₂₀H₂₃O₈N₄S₂ [M+H]⁺ 511.0952; found 511.0951.

L-cystine *p*-bromobenzyl ester *p*-toluenesulphonate, **67**

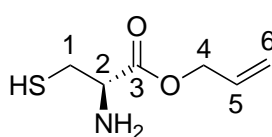


Quantitative yield (4.8 g); white solid, m.p. 194 °C; $[\alpha]_D^{25} = -12.9$ ($c = 0.30$, MeOH); $\nu_{\max}/\text{cm}^{-1}$ (neat) 2923 (br. s, N-H), 1745 (s, C=O); ¹H NMR

(methanol-*d*₄, 400 MHz): δ 7.70 (d, $J = 8.2$ Hz, 4H, H2'), 7.51 (d, $J = 8.4$ Hz, 4H, H7), 7.34 (d, $J = 8.4$ Hz, 4H, H6), 7.23 (d, $J = 7.8$ Hz, 4H, H3'), 5.24 (d, $J = 2.4$ Hz, 4H, H4), 4.45 – 4.51 (m, 2H, H2), 3.27 – 3.35 (m, 4H, H1, obscured by solvent peak), 2.37 (s, 6H, H5'); ¹³C NMR (methanol-*d*₄, 101 MHz): δ 168.8 (C3), (C1' not seen), 141.8 (C4'), 135.4 (C5), 132.9 (C7), 131.7 (C6), 129.8 (C2'), 127.0 (C3'), 123.7 (C8), 68.8 (C4), 53.0 (C2), 37.9 (C1), 21.3 (C5'); m/z (ESI⁺) 576 ([M+H]⁺, 50%, M as free base), 578 ([M+H]⁺, 100%), 580 ([M+H]⁺, 50%); HRMS (ESI⁺) m/z calcd for C₂₀H₂₃O₄N₂Br₂S₂ [M+H]⁺ 576.9437, 578.9416, 580.9395; found 576.9462, 578.9439 and 580.9417.

L-cysteine esters **68-74** were synthesised from their respective L-cysteine diester *p*-toluenesulphonate salt **61-67** (7.5 mmol) following general procedure **M** (section 6.4.2).

L-cysteine allyl ester, **68**

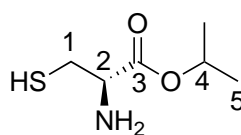


Yield (2.2 g, 82%); pale yellow oil; $R_f = 0.23$ (EtOAc: MeOH; 7 : 1); $[\alpha]_D^{25} = -76.0$ ($c = 1.0$, CHCl₃); $\nu_{\max}/\text{cm}^{-1}$ 3299 (br. s, N-H), 1734 (s,

C=O); ¹H NMR (CDCl₃, 400 MHz): δ 5.85 – 5.98 (m, 1H, H5), 5.33 (d, $J = 17.2$ Hz, 1H, H6_B),

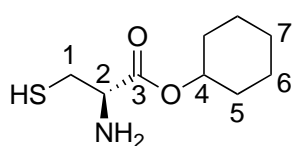
5.26 (d, $J = 10.4$ Hz, 1H, H_{6A}), 4.63 (d, $J = 5.8$ Hz, 2H, H₄), 3.80 – 3.88 (m, 1H, H₂), 3.11 – 3.18 (m, 1H, H_{1B}), 2.89 – 2.98 (m, 1H, H_{1A}), 2.17 (br. s, 3H, SH, NH₂); ¹³C NMR (CDCl₃, 101 MHz): δ 173.5 (C₃), 131.7 (C₅), 119.1 (C₆), 66.2 (C₄), 53.7 (C₂), 43.7 (C₁); m/z (ESI⁺) 162 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for C₆H₁₂O₂NS [M+H]⁺ 162.0583; found 162.0583.

L-cysteine isopropyl ester, 69



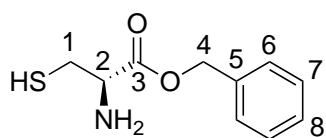
Yield (1.5 g, 65%); pale yellow oil; $R_f = 0.20$ (EtOAc: MeOH; 7 : 1); $[\alpha]_D^{25} = -6.89$ ($c = 1.0$, CHCl₃); $\nu_{\max}/\text{cm}^{-1}$ 3307 (br. s, N-H), 1733 (s, C=O); ¹H NMR (CDCl₃, 400 MHz): δ 5.04 (sept, $J = 6.3$ Hz, 1H, H₄), 3.73 (dd, $J = 7.9, 4.6$ Hz, 1H, H₂), 3.12 (dd, $J = 13.5, 4.6$ Hz, 1H, H_{1B}), 2.88 (dd, $J = 13.5, 7.9$ Hz, 1H, H_{1A}), 1.90 (br. s, 3H, SH, NH₂), 1.26 (s, 3H, H_{5B}), 1.24 (s, 3H, H_{5A}); ¹³C NMR (CDCl₃, 101 MHz): δ 173.3 (C₃), 69.2 (C₄), 53.9 (C₂), 43.9 (C₁), 21.9 (C₅); m/z (ESI⁺) 164 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for C₆H₁₄O₂NS [M+H]⁺ 164.0740; found 164.0740.

L-cysteine cyclohexyl ester, 70



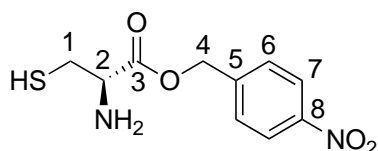
Yield (2.0 g, 71%); colourless oil; $R_f = 0.20$ (EtOAc: MeOH; 8 : 1); $[\alpha]_D^{25} = -34.5$ ($c = 0.90$, CHCl₃); $\nu_{\max}/\text{cm}^{-1}$ 3370 (br. s, N-H), 1731 (s, C=O); ¹H NMR (CDCl₃, 400 MHz): δ 4.74 – 4.88 (m, 1H, H₄), 3.76 (dd, $J = 7.8, 4.6$ Hz, 1H, H₂), 3.14 (dd, $J = 13.5, 4.6$ Hz, 1H, H_{1B}), 2.90 (dd, $J = 13.5, 7.8$ Hz, 1H, H_{1A}), 1.93 (br. s, 3H, SH, NH₂), 1.79 – 1.89 (m, 2H, H₅), 1.64 – 1.77 (m, 2H, H₅), 1.21 – 1.58 (m, 6H, H₆, H₇); ¹³C NMR (CDCl₃, 101 MHz): δ 173.3 (C₃), 74.0 (C₄), 54.0 (C₂), 44.0 (C₁), 31.6 (C₅), 25.4 (C₇), 23.8 (C₆); m/z (ESI⁺) 204 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for C₉H₁₈O₂NS [M+H]⁺ 204.1053; found 204.1055.

L-cysteine benzyl ester, 71



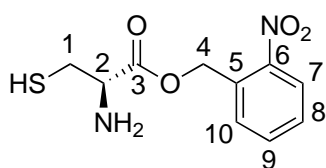
Yield (2.9 g, 83%); yellow oil; $R_f = 0.40$ (EtOAc: MeOH; 9 : 1); $[\alpha]_D^{25} = -38.0$ ($c = 0.96$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 3304 (br. s, N-H), 1737 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ 7.35 (br. s, 5H, H6, H7, H8), 5.16 (s, 2H, H4), 3.82 (dd, $J = 7.6, 4.6$ Hz, 1H, H2), 3.12 (dd, $J = 13.6, 4.6$ Hz, 1H, H1_B), 2.90 (dd, $J = 13.6, 7.6$ Hz, 1H, H1_A), 1.85 (br. s, 3H, SH, NH₂); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ 173.7 (C3), 135.5 (C5), 128.8 (C7), 128.6 (C8), 128.5 (C6), 67.3 (C4), 53.8 (C2), 43.7 (C1); m/z (ESI⁺) 212 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for $\text{C}_{10}\text{H}_{14}\text{O}_2\text{NS}$ [M+H]⁺ 212.0740; found 212.0745.

L-cysteine *p*-nitrobenzyl ester, 72



Yield (2.1 g, 50%); yellow oil; $R_f = 0.15$ (EtOAc: MeOH; 9 : 1); $[\alpha]_D^{25} = -5.13$ ($c = 0.54$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 3374 (br. s, N-H), 1742 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ 8.24 (d, $J = 8.8$ Hz, 2H, H7), 7.54 (d, $J = 8.8$ Hz, 2H, H6), 5.23 – 5.32 (m, 2H, H4), 3.88 (dd, $J = 7.2, 5.0$ Hz, 1H, H2), 3.13 (dd, $J = 13.6, 5.0$ Hz, 1H, H1_B), 2.97 (dd, $J = 13.6, 7.2$ Hz, 1H, H1_A), 1.72 (br. s, 3H, SH, NH₂); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ 173.4 (C3), 148.0 (C8), 142.7 (C5), 128.8 (C7), 124.1 (C6), 65.7 (C4), 56.1 (C2), 43.7 (C1); m/z (ESI⁺) 257 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for $\text{C}_{10}\text{H}_{13}\text{O}_4\text{N}_2\text{S}$ [M+H]⁺ 257.0951; found 257.0952.

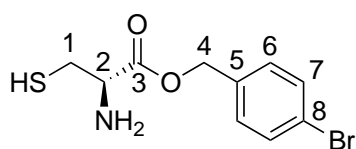
L-cysteine *o*-nitrobenzyl ester, 73



Yield (4.0 g, 94%); pale yellow oil; $R_f = 0.70$ (EtOAc: MeOH; 9 : 1); $[\alpha]_D^{25} = -15.2$ ($c = 1.37$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 3297 (br. s, N-H), 1742 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ 8.10 (dd, $J = 8.1, 1.2$ Hz, 1H, H7), 7.66 (td, $J = 7.6, 1.3$ Hz, 1H, H9), 7.63 – 7.56 (m, 1H, H8), 7.56 – 7.47 (m,

1H, H10), 5.56 (app d, $J = 3.8$ Hz, 2H, H4), 3.80 – 3.69 (m, 1H, H2), 2.89 (dd, $J = 5.2, 3.5$ Hz, 2H, H1), 1.84 (br. s, 3H, SH, NH₂); ¹³C NMR (CDCl₃, 101 MHz): δ 173.3 (C3), 148.0 (C6), 133.9 (C10), 131.4 (C5), 129.7 (C9), 129.3 (C8), 125.3 (C7), 64.0 (C4), 56.1 (C2), 43.6 (C1); m/z (ESI⁺) 257 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for C₁₀H₁₃O₄N₂S [M+H]⁺ 257.0951; found 257.0952.

L-cysteine *p*-bromobenzyl ester, **74**



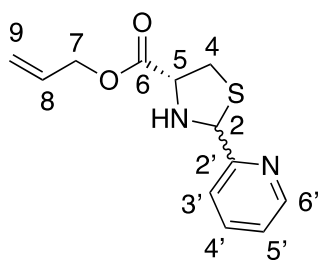
Yield (2.5 g, 53%); white solid, m.p. 129 °C; $R_f = 0.20$ (100%

EtOAc); $[\alpha]_D^{25} = -38.0$ ($c = 1.0$, CHCl₃); $\nu_{\max}/\text{cm}^{-1}$ (neat) 1737 (s,

C=O); ¹H NMR (CDCl₃, 400 MHz): δ 7.53 – 7.46 (m, 2H, H7), 7.27 – 7.20 (m, 2H, H6), 5.15 – 5.08 (m, 2H, H4), 3.82 (dd, $J = 7.5, 4.7$ Hz, 1H, H2), 3.10 (dd, $J = 13.7, 4.7$ Hz, 1H, H1_B), 2.91 (dd, $J = 13.7, 7.5$ Hz, 1H, H1_A), 2.09 (br. s, 3H, SH, NH₂); ¹³C NMR (CDCl₃, 101 MHz): δ 173.5 (C3), 134.4 (C5), 132.0 (C7), 130.2 (C6), 122.8 (C8), 66.5 (C4), 53.8 (C2), 43.6 (C1); m/z (ESI⁺) 290 and 292 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for C₁₀H₁₃O₂NBrS [M+H]⁺ 289.9845 and 291.9824; found 289.9846 and 291.9826.

Thiazolidines **75-81** were synthesised from the respective L-cysteine esters **68-74** with the respective aldehyde by general procedure **A** (section 6.2.1)

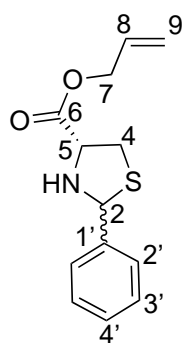
(5*R*)-5-Alloxycarbonyl-2-(pyridin-2-yl)-1,3-thiazolidine, **75a**



Synthesised from **68** (1 eqv. 11 mmol) with pyridine-2-carboxaldehyde; yield (2.6 g, 95%); greenish yellow oil; separable mixture of 1 : 1 *cis* and *trans* diastereomers; $R_f =$

0.61 (*cis*), 0.35 (*trans*) (petrol : EtOAc; 1 : 1); $[\alpha]_D^{25} = -47.9$ ($c = 1.04$, CHCl_3), *cis*; $[\alpha]_D^{25} = -39.1$ ($c = 0.63$, CHCl_3), *trans*; $\nu_{\text{max}}/\text{cm}^{-1}$ 3293 (br. s, N-H), 1739 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): *cis*: δ (NH not observed), 8.63 (d, $J = 4.8$ Hz, 1H, H6'), 7.66 (app td, $J = 7.7, 1.8$ Hz, 1H, H4'), 7.29 (d, $J = 7.7$ Hz, 1H, H3'), 7.23 (dd, $J = 7.7, 4.8$ Hz, 1H, H5'), 5.89 – 6.01 (m, 1H, H8), 5.65 (d, $J = 8.7$ Hz, 1H, H2), 5.37 (dd, $J = 17.2, 1.5$ Hz, 1H, H9_A), 5.28 (dd, $J = 10.4, 1.5$ Hz, 1H, H9_A), 4.72 (d, $J = 5.8$ Hz, 2H, H7), 4.01 – 4.10 (m, 1H, H5), 3.46 (dd, $J = 10.0, 6.6$ Hz, 1H, H4_B), 3.10 (app t, $J = 9.8$ Hz, 1H, H4_A); *trans*: δ 8.58 (d, $J = 4.8$ Hz, 1H, H6'), 7.66 (app td, $J = 7.7, 1.8$ Hz, 1H, H4'), 7.34 (d, $J = 7.7$ Hz, 1H, H3'), 7.20 (dd, $J = 7.7, 4.8$ Hz, 1H, H5'), 5.90 – 6.01 (m, 1H, H8), 5.86 (d, $J = 6.2$ Hz, 1H, H2), 5.37 (dd, $J = 17.2, 1.4$ Hz, 1H, H9_B), 5.29 (dd, $J = 10.4, 1.4$ Hz, 1H, H9_A), 4.70 (d, $J = 5.8$ Hz, 2H, H7), 4.53 – 4.60 (m, 1H, H5), 3.66 (br. s, 1H, NH), 3.40 (dd, $J = 10.6, 6.8$ Hz, 1H, H4_B), 3.35 (dd, $J = 10.6, 4.4$ Hz, 1H, H4_A); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): *cis*: δ 170.5 (C6), 156.8 (C2'), 150.0 (C6'), 136.9 (C4'), 131.6 (C8), 123.5 (C3'), 122.2 (C5'), 119.3 (C9), 71.7 (C5), 66.4 (C7), 66.2 (C2), 39.5 (C4); *trans*: δ 171.5 (C6), 158.8 (C2'), 149.8 (C6'), 136.9 (C4'), 131.8 (C8), 123.1 (C3'), 121.7 (C5'), 119.1 (C9), 71.2 (C5), 66.2 (C7), 65.8 (C2), 38.9 (C4); m/z (ESI⁺) 251 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for $\text{C}_{12}\text{H}_{15}\text{O}_2\text{N}_2\text{S}$ [M+H]⁺ 251.0849; found 251.0849.

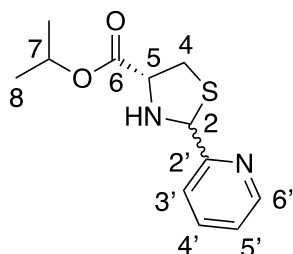
(5R)-5-Alloxycarbonyl-2-phenyl-1,3-thiazolidine, 75e



Synthesised from **68** (1 eqv. 13.6 mmol) with benzaldehyde; yield (2.3 g, 68%); yellow oil; an inseparable mixture of 1.8 : 1 *cis* and *trans* diastereomers; $R_f = 0.44$ (petrol : EtOAc; 9 : 1); $\nu_{\text{max}}/\text{cm}^{-1}$ 3309 (br. s, N-H), 1736 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): major *cis* isomer: δ (NH not observed), 7.47 – 7.55 (m, 2H, H2'), 7.31 – 7.40 (m, 3H, H4' and H3'), 5.87 – 6.03 (m, 1H, H8), 5.57 (s, 1H, H2), 5.36 (dd, $J = 17.2, 1.3$ Hz, 1H, H9_B), 5.29 (dd, $J = 10.4,$

1.3 Hz, 1H, H9_A), 4.66 – 4.75 (m, 2H, H7), 3.96 – 4.06 (m, 1H, H5), 3.49 (dd, $J = 10.3, 7.1$ Hz, 1H, H4_B), 3.13 (dd, $J = 10.3, 8.9$ Hz, 1H, H4_A); minor *trans* isomer: δ (NH not observed), 7.47 – 7.55 (m, 2H, H2'), 7.31 – 7.40 (m, 3H, H4' and H3'), 5.87 – 6.03 (m, 1H, H8), 5.83 (s, 1H, H2), 5.36 (dd, $J = 17.2, 1.3$ Hz, 1H, H9_B), 5.29 (dd, $J = 10.4, 1.3$ Hz, 1H, H9_A), 4.66 – 4.75 (m, 2H, H7), 4.20 – 4.28 (m, 1H, H5), 3.41 (dd, $J = 10.6, 7.1$ Hz, 1H, H4_B), 3.22 (dd, $J = 10.6, 5.8$ Hz, 1H, H4_A); ¹³C NMR (CDCl₃, 101 MHz): major *cis* isomer: δ 171.0 (C6), 138.3 (C1'), 131.5 (C8), 128.8 (C3'), 128.6 (C4'), 127.6 (C2'), 119.3 (C9), 72.8 (C5), 66.3 (C7), 65.8 (C2), 39.4 (C4); minor *trans* isomer: δ 171.6 (C6), 141.3 (C1'), 131.6 (C8), 128.8 (C3'), 128.0 (C4'), 127.0 (C2'), 119.2 (C9), 70.9 (C5), 66.2 (C7), 64.5 (C2), 38.3 (C4); m/z (ESI⁻) 248 ([M-H]⁻, 100%); HRMS (ESI⁻) m/z calcd for C₁₃H₁₄O₂NS [M-H]⁻ 248.0740; found 248.0740.

(5*R*)-5-Isopropoxycarbonyl-2-(pyridin-2-yl)-1,3-thiazolidine, 76a

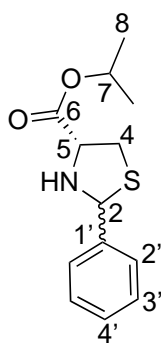


Synthesised from **69** (1 eqv. 10 mmol) with pyridine-2-carboxaldehyde; yield (2.2 g, 85%); yellow oil; separable mixture of 1 : 1 *cis* and *trans* diastereomers; $R_f = 0.45$ (*cis*), 0.24 (*trans*) (petrol : EtOAc; 1 : 1); $[\alpha]_D^{25} = -67.4$ ($c = 1.18$, CHCl₃), *cis*; $[\alpha]_D^{25} = -$

64.1 ($c = 1.31$, CHCl₃), *trans*; ν_{max}/cm^{-1} 3294 (br. s, N-H), 1734 (s, C=O); ¹H NMR (CDCl₃, 400 MHz): *cis*: δ 8.62 (d, $J = 4.8$ Hz, 1H, H6'), 7.66 (app td, $J = 7.7, 1.8$ Hz, 1H, H4'), 7.28 (d, $J = 7.7$ Hz, 1H, H3'), 7.23 (dd, $J = 7.7, 4.8$ Hz, 1H, H5'), 5.64 (d, $J = 10.7$ Hz, 1H, H2), 5.14 (sept, $J = 6.3$ Hz, 1H, H7), 3.97 (dd, $J = 10.4, 9.4$ Hz, 1H, H5), 3.81 (br. s, 1H, NH), 3.43 (dd, $J = 10.4, 6.6$ Hz, 1H, H4_B), 3.06 (app t, $J = 9.8$ Hz, 1H, H4_A), 1.29 (app dd, $J = 6.3, 4.0$ Hz, 6H, H8); *trans*: δ (NH not observed), 8.57 (d, $J = 4.8$ Hz, 1H, H6'), 7.65 (app td, $J = 7.7, 1.8$ Hz, 1H, H4'), 7.34 (d, $J = 7.7$ Hz, 1H, H3'), 7.19 (dd, $J = 7.7, 4.8$ Hz, 1H, H5'), 5.86 (s, 1H, H2), 5.11 (sept, $J = 6.3$ Hz, 1H, H7), 4.46 (dd, $J = 6.9, 4.6$ Hz, 1H, H5), 3.37 (dd, $J = 10.6, 6.9$ Hz,

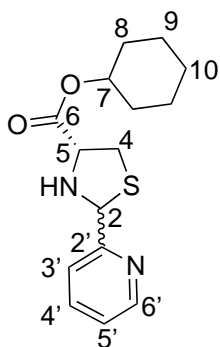
1H, H4_B), 3.27 (dd, *J* = 10.6, 4.6 Hz, 1H, H4_A), 1.29 (app dd, *J* = 6.3, 4.5 Hz, 6H, H8); ¹³C NMR (CDCl₃, 101 MHz): *cis*: δ 170.3 (C6), 156.9 (C2'), 150.0 (C6'), 136.8 (C4'), 123.4 (C3'), 122.2 (C5'), 71.8 (C5), 69.4 (C7), 66.6 (C2), 39.6 (C4), 21.9 (C8); *trans*: δ 171.3 (C6), 159.2 (C2'), 149.7 (C6'), 136.9 (C4'), 123.0 (C3'), 121.5 (C5'), 71.3 (C5), 69.3 (C7), 65.9 (C2), 39.0 (C4), 21.9 (C8); *m/z* (ESI⁺) 253 ([M+H]⁺, 100%); HRMS (ESI⁺) *m/z* calcd for C₁₂H₁₇O₂N₂S [M+H]⁺ 253.1005; found 253.1007.

(5*R*)-5-Isopropoxycarbonyl-2-phenyl-1,3-thiazolidine, 76e



Synthesised from **69** (1 eqv. 10 mmol) with benzaldehyde; yield (2.0 g, 83%); pale yellow oil; an inseparable mixture of 1.9 : 1 *cis* and *trans* diastereomers; *R_f* = 0.36 (petrol : EtOAc; 9 : 1); *v*_{max}/cm⁻¹ 3312 (br. s, N-H), 1730 (s, C=O); ¹H NMR (CDCl₃, 400 MHz): major *cis* isomer: δ 7.49 (d, *J* = 6.6 Hz, 2H, H2'), 7.27 – 7.35 (m, 3H, H4' and H3'), 5.52 (s, 1H, H2), 5.08 (sept, *J* = 6.3 Hz, 1H, H7), 3.89 (dd, *J* = 8.9, 7.2 Hz, 1H, H5), 3.41 (dd, *J* = 10.3, 7.2 Hz, 1H, H4_B), 3.05 (dd, *J* = 10.2, 8.9 Hz, 1H, H4_A), 2.72 (br. s, 1H, NH), 1.24 (app dd, *J* = 6.3, 2.1 Hz, 6H, H8); minor *trans* isomer: δ 7.45 (d, *J* = 7.2 Hz, 2H, H2'), 7.27 – 7.35 (m, 3H, H4' and H3'), 5.80 (s, 1H, H2), 5.08 (sept, *J* = 6.3 Hz, 1H, H7), 4.05 – 4.12 (m, 1H, H5), 3.32 (dd, *J* = 10.5, 7.1 Hz, 1H, H4_B), 3.10 (dd, *J* = 10.5, 6.0 Hz, 1H, H4_A), 2.72 (br. s, 1H, NH), 1.24 (dd, *J* = 6.3, 2.1 Hz, 6H, H8); ¹³C NMR (CDCl₃, 101 MHz): major *cis* isomer: δ 170.6 (C6), 138.2 (C1'), 128.6 (C3'), 127.4 (C4'), 126.9 (C2'), 72.7 (C5), 69.3 (C7), 65.8 (C2), 39.3 (C4), 21.7 (C8); minor *trans* isomer: δ 171.1 (C6), 141.5 (C1'), 128.3 (C3'), 127.7 (C4'), 126.9 (C2'), 70.9 (C5), 69.1 (C7), 64.4 (C2), 38.2 (C4), 21.7 (C8); *m/z* (ESI⁺) 252 ([M+H]⁺, 100%); HRMS (ESI⁺) *m/z* calcd for C₁₃H₁₈O₂NS [M+H]⁺ 252.1053; found 252.1053.

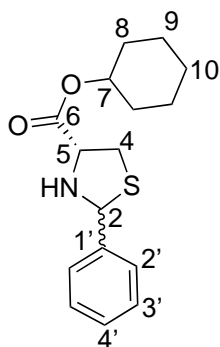
(5R)-5-Cyclohexyloxycarbonyl-2-(pyridin-2-yl)-1,3-thiazolidine, 77a



Synthesised from **70** (1 eqv. 4.5 mmol) with pyridine-2-carboxaldehyde; yield (0.91 g, 69%); yellow oil; separable mixture of 1.1 : 1 *cis* and *trans* diastereomers; $R_f = 0.53$ (*cis*), 0.31 (*trans*) (petrol : EtOAc; 3 : 2); $[\alpha]_D^{25} = -25.9$ ($c = 0.20$, CHCl_3), *cis*; $[\alpha]_D^{25} = -35.4$ ($c = 27$, CHCl_3), *trans*; $\nu_{\text{max}}/\text{cm}^{-1}$ 3294 (br. s, N-H), 1733 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz):

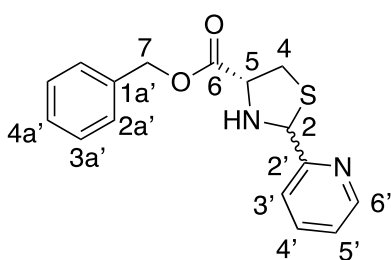
cis: δ (NH not observed), 8.59 (d, $J = 4.8$ Hz, 1H, H6'), 7.63 (app td, $J = 7.7, 1.8$ Hz, 1H, H4'), 7.27 (d, $J = 7.7$ Hz, 1H, H3'), 7.11 (app ddd, $J = 7.7, 4.8, 1.0$ Hz, 1H, H5'), 5.64 (s, 1H, H2), 4.94 – 4.82 (m, 1H, H7), 4.03 – 3.92 (m, 1H, H5), 3.42 (dd, $J = 10.0, 6.6$ Hz, 1H, H4_B), 3.05 (app t, $J = 9.7$ Hz, 1H, 1H, H4_A), 1.89 – 1.82 (m, 2H, H8), 1.75 – 1.67 (m, 2H, H8), 1.54 – 1.20 (m, 6H, H9 and H10); *trans*: δ (NH not observed), 8.49 (d, $J = 4.8$ Hz, 1H, H6'), 7.57 (app td, $J = 7.7, 1.8$ Hz, 1H, H4'), 7.27 (d, $J = 7.7$ Hz, 1H, H3'), 7.11 (app ddd, $J = 7.7, 4.8, 1.1$ Hz, 1H, H5'), 5.81 (s, 1H, H2), 4.88 – 4.68 (m, 1H, H7), 4.41 (dd, $J = 6.9, 4.6$ Hz, 1H, H5), 3.30 (dd, $J = 10.5, 6.9$ Hz, 1H, H4_B), 3.20 (dd, $J = 10.5, 4.6$ Hz, 1H, H4_A), 1.87 – 1.75 (m, 2H, H8), 1.71 – 1.62 (m, 2H, H8), 1.49 – 1.18 (m, 6H, H9 and H10); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): *cis*: δ 169.9 (C6), 159.6 (C2'), 149.6 (C6'), 136.5 (C4'), 123.1 (C3'), 121.9 (C5'), 73.7 (C7), 71.5 (C5), 66.3 (C2), 39.3 (C4), 31.2 (C8), 25.1 (C10), 23.4 (C9); *trans*: δ 171.0 (C6), 159.1 (C2'), 149.5 (C6'), 136.7 (C4'), 122.7 (C3'), 121.3 (C5'), 73.7 (C7), 71.2 (C5), 65.7 (C2), 38.8 (C4), 31.4 (C8), 25.2 (C10), 23.6 (C9); m/z (ESI⁺) 293 ($[\text{M}+\text{H}]^+$, 100%); HRMS (ESI⁺) m/z calcd for $\text{C}_{15}\text{H}_{21}\text{O}_2\text{N}_2\text{S}$ $[\text{M}+\text{H}]^+$ 293.1318; found 293.1319.

(5*R*)-5-Cyclohexyloxycarbonyl-2-phenyl-1,3-thiazolidine, 77e



Synthesised from **70** (1 eqv. 6.9 mmol) with benzaldehyde; yield (1.4 g, 70%); pale yellow oil; an inseparable mixture of 1.9 : 1 *cis* and *trans* diastereomers; $R_f = 0.22$ (petrol : EtOAc; 19 : 1); $\nu_{\max}/\text{cm}^{-1}$ 3312 (br. s, N-H), 1732 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): major *cis* isomer: δ 7.48 (d, $J = 7.6$ Hz, 2H, H2'), 7.27 – 7.35 (m, 3H, H4' and H3'), 5.52 (s, 1H, H2), 4.75 – 4.92 (m, 1H, H7), 3.91 (dd, $J = 8.9, 7.2$ Hz, 1H, H5), 3.42 (dd, $J = 10.3, 7.2$ Hz, 1H, H4_B), 3.05 (dd, $J = 10.3, 8.9$ Hz, 1H, H4_A), 2.70 (br. s, 1H, NH), 1.78 – 1.89 (m, 2H, H8), 1.63 – 1.74 (m, 2H, H8), 1.16 – 1.56 (m, 6H, H9 and H10); minor *trans* isomer: δ 7.44 (d, $J = 7.5$ Hz, 2H, H2'), 7.27 – 7.35 (m, 3H, H4' and H3'), 5.80 (s, 1H, H2), 4.75 – 4.92 (m, 1H, H7), 4.06 – 4.16 (m, 1H, H5), 3.34 (dd, $J = 10.5, 7.1$ Hz, 1H, H4_B), 3.11 (dd, $J = 10.5, 6.0$ Hz, 1H, H4_A), 2.70 (br. s, 1H, NH), 1.78 – 1.89 (m, 2H, H8), 1.63 – 1.74 (m, 2H, H8), 1.16 – 1.56 (m, 6H, H9 and H10); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): major *cis* isomer: δ 170.7 (C6), 138.3 (C1'), 128.7 (C3'), 127.5 (C4'), 127.0 (C2'), 74.2 (C7), 72.8 (C5), 66.0 (C2), 39.5 (C4), 31.5 (C8), 25.3 (C10), 23.7 (C9); minor *trans* isomer: δ 171.2 (C6), 141.6 (C1'), 128.4 (C3'), 127.8 (C4'), 127.0 (C2'), 74.0 (C7), 70.9 (C5), 64.6 (C2), 38.4 (C4), 31.5 (C8), 25.3 (C10), 23.7 (C9); m/z (ESI⁺) 292 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for $\text{C}_{16}\text{H}_{22}\text{O}_2\text{NS}$ [M+H]⁺ 292.1366; found 292.1365.

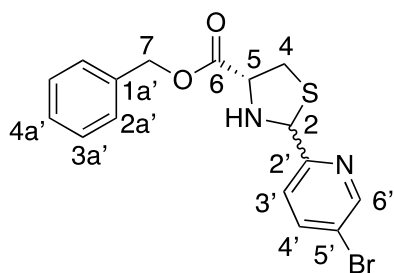
(5*R*)-5-Benzoyloxycarbonyl-2-(pyridin-2-yl)-1,3-thiazolidine, 78a



Synthesised from **71** (1 eqv. 7.4 mmol) with pyridine-2-carboxaldehyde; yield (1.0 g, 45%); pale yellow oil; an inseparable mixture of 1 : 1 *cis* and *trans* diastereomers;

$R_f = 0.43$ (petrol : EtOAc; 1 : 1); $\nu_{\max}/\text{cm}^{-1}$ 3288 (br. s, N-H), 1736 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): *cis*: δ 8.56 (d, $J = 5.3$ Hz, 1H, H6'), 7.58 (app td, $J = 7.8, 1.8$ Hz, 1H, H4'), 7.27 – 7.31 (m, 5H, H4a', H3a', H2a'), 7.24 (d, $J = 7.8$ Hz, 1H, H3'), 7.14 – 7.19 (m, 1H, H5'), 5.61 (s, 1H, H2), 5.21 (s, 2H, H7), 4.03 (dd, $J = 9.6, 6.7$ Hz, 1H, H5), 3.81 (br. s, 1H, NH), 3.40 (dd, $J = 10.1, 6.7$ Hz, 1H, H4_B), 3.05 (app t, $J = 9.8$ Hz, 1H, H4_A); *trans*: δ (NH not observed), 8.51 (d, $J = 4.7$ Hz, 1H, H6'), 7.58 (app td, $J = 7.7, 1.8$ Hz, 1H, H4'), 7.27 – 7.34 (m, 6H, H4a', H3a', H2a' and H3'), 7.12 (dd, $J = 7.7, 5.2$ Hz, 1H, H5'), 5.83 (s, 1H, H2), 5.18 (s, 2H, H7), 4.52 (dd, $J = 6.8, 4.4$ Hz, 1H, H5), 3.32 (dd, $J = 10.6, 6.8$ Hz, 1H, H4_B), 3.27 (dd, $J = 10.6, 4.4$ Hz, 1H, H4_A); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): *cis*: δ 170.5 (C6), 156.5 (C2'), 149.6 (C6'), 136.7 (C4'), 135.1 (C1a'), 128.4 (C4a'), 128.3 (C4a'), 128.2 (C2a'), 123.3 (C3'), 122.0 (C5'), 71.4 (C5), 67.1 (C7), 66.0 (C2), 39.2 (C4); *trans*: δ 171.4 (C6), 158.6 (C2'), 149.4 (C6'), 136.7 (C4'), 135.3 (C1a'), 128.5 (C3a'), 128.3 (C4a'), 128.1 (C2a'), 122.8 (C3'), 121.4 (C5'), 70.9 (C5), 67.0 (C7), 65.5 (C2), 38.5 (C4); m/z (ESI⁺) 301 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for $\text{C}_{16}\text{H}_{17}\text{O}_2\text{N}_2\text{S}$ [M+H]⁺ 301.1005; found 301.1004.

(5R)-5-Benzyloxycarbonyl-2-(5-bromopyridin-2-yl)-1,3-thiazolidine, 78c

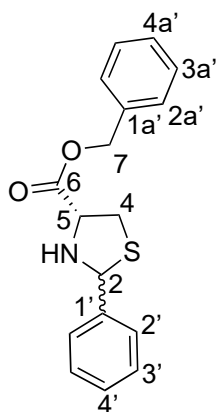


Synthesised from **71** (1 eqv. 5.6 mmol) with 5-bromopyridine-2-carbaldehyde; yield (1.8 g, 85%); yellow oil; an inseparable mixture of 1 : 1 *cis* and *trans* diastereomers; $R_f = 0.25$ (petrol : EtOAc; 17 : 3); $\nu_{\max}/\text{cm}^{-1}$

3292 (br. s, N-H), 1737 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ 8.66 (d, $J = 2.3$ Hz, 1H, H6' *cis*), 8.60 (d, $J = 2.3$ Hz, 1H, H6' *trans*), 7.77 (app ddd, $J = 8.3, 6.9, 2.4$ Hz, 2H, H4' *cis* and *trans*), 7.41 – 7.30 (m, 10H, H2a', H3a' and H4a', *cis* and *trans*), 7.24 (d, $J = 8.3$ Hz, 1H, H3' *trans*), 7.19 (d, $J = 8.3$ Hz, 1H, H3' *cis*), 5.82 (s, 1H, H2 *trans*), 5.61 (s, 1H, H2 *cis*), 5.26 (s,

2H, H7 *trans*), 5.23 (s, 2H, H7 *cis*), 4.50 (dd, $J = 6.8, 4.7$ Hz, 1H, H5 *trans*), 4.06 (dd, $J = 9.5, 6.7$ Hz, 1H, H5 *cis*), 3.75 (br. s, 2H, NH, *cis* and *trans*), 3.43 (dd, $J = 10.1, 6.7$ Hz, 1H, H4_B *cis*), 3.36 (dd, $J = 10.6, 6.8$ Hz, 1H, H4_B *trans*), 3.27 (dd, $J = 10.6, 4.7$ Hz, 1H, H4_A *trans*), 3.07 (app t, $J = 9.8$ Hz, 1H, H4_A *cis*); ¹³C NMR (CDCl₃, 101 MHz): δ 171.4, 170.6 (C6), 158.0, 155.6 (C2'), 151.0, 150.7 (C6'), 139.4 (C4'), 135.4, 135.3 (C1a'), 128.8 (C4a'), 128.7, 128.6 (C4a'), 128.4, 128.4 (C2a'), 123.3, 122.6 (C3'), 120.2, 119.6 (C5'), 71.0, 70.5 (C5), 67.4, 67.3 (C7), 66.3, 65.6 (C2), 39.5, 38.8 (C4); m/z (ESI⁺) 379 and 381 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for C₁₆H₁₆O₂N₂BrS [M+H]⁺ 379.0110 and 381.0089; found 379.0112 and 381.0091.

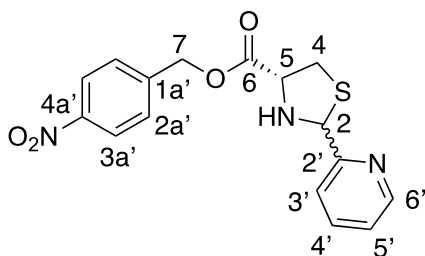
(5R)-5-Benzyloxycarbonyl-2-phenyl-1,3-thiazolidine, 78e



Synthesised from **71** (1 eqv. 8.7 mmol) with benzaldehyde; yield (1.3 g, 50%); pale yellow oil; an inseparable mixture of 1.8 : 1 *cis* and *trans* diastereomers; $R_f = 0.72$ (petrol : EtOAc; 7 : 3); $\nu_{\max}/\text{cm}^{-1}$ 3031 (br. s, N-H), 1736 (s, C=O); ¹H NMR (CDCl₃, 400 MHz): major *cis* isomer: δ 7.53 (d, $J = 7.8$ Hz, 2H, H2'), 7.32 – 7.40 (m, 8H, H4', H4a', H3', H3a', H2a'), 5.57 (s, 1H, H2), 5.25 (s, 2H, H7), 3.99 – 4.08 (m, 1H, H5), 3.48 (dd, $J = 10.3, 7.1$ Hz, 1H, H4_B), 3.12 (dd, $J = 10.3, 8.9$ Hz, 1H, H4_A), 2.72 (br. s, 1H, NH); minor *trans* isomer: δ 7.48 (d, $J = 7.5$ Hz, 2H, H2'), 7.32 – 7.40 (m, 8H, H4', H4a', H3', H3a', H2a'), 5.83 (s, 1H, H2), 5.23 (app d, $J = 2.2$ Hz, 2H, H7), 4.22 – 4.29 (m, 1H, H5), 3.41 (dd, $J = 10.6, 7.1$ Hz, 1H, H4_B), 3.22 (dd, $J = 10.6, 5.8$ Hz, 1H, H4_A), 2.72 (br. s, 1H, NH); ¹³C NMR (CDCl₃, 101 MHz): major *cis* isomer: δ 171.2 (C6), 138.3 (C1'), 135.2 (C1a'), 128.8 (C3'), 128.7 (C3a'), 128.5 (C4a'), 128.4 (C2a'), 127.6 (C4'), 127.1 (C2'), 72.8 (C5), 67.5 (C7), 65.8 (C2), 39.4 (C4); minor *trans* isomer: δ 171.7 (C6), 141.3 (C1'), 135.4 (C1a'), 128.8 (C3'), 128.7 (C3a'), 128.6

(C4a'), 128.0 (C2a'), 127.6 (C4'), 127.1 (C2'), 70.9 (C5), 67.4 (C7), 64.5 (C2), 38.2 (C4); m/z (ESI⁺) 300 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for C₁₇H₁₈O₂NS [M+H]⁺ 300.1064; found 300.1055.

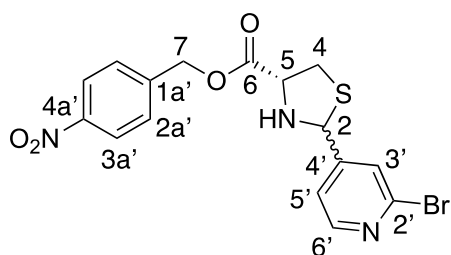
(5R)-5-(4-nitrobenzyloxycarbonyl)-2-(pyridin-2-yl)-1,3-thiazolidine, 79a



Synthesised from **72** (1 eqv. 7.8 mmol) with pyridine-2-carbaldehyde; yield (2.0 g, 74%); yellow oil; separable 1 : 1 *cis* and *trans* diastereomers; R_f = 0.38 (*cis*), 0.15 (*trans*) (petrol : EtOAc; 1 : 1); $[\alpha]_D^{25}$ = -52.5 (c = 0.97,

CHCl₃), *cis*; $[\alpha]_D^{25}$ = -56.0 (c = 1.10, CHCl₃), *trans*; $\nu_{\max}/\text{cm}^{-1}$ 3287 (br. s, N-H), 1743 (s, C=O); ¹H NMR (CDCl₃, 400 MHz): *cis*: δ (NH not observed), 8.63 (d, J = 4.8 Hz, 1H, H6'), 8.24 (d, J = 8.8 Hz, 2H, H3a'), 7.68 (app td, J = 7.7, 1.8 Hz, 1H, H4'), 7.56 (d, J = 8.8 Hz, 2H, H2a'), 7.29 (d, J = 7.7 Hz, 1H, H3'), 7.25 (dd, J = 7.7, 4.8 Hz, 1H, H5'), 5.66 (s, 1H, H2), 5.35 (s, 2H, H7), 4.11 (dd, J = 9.3, 6.9 Hz, 1H, H5), 3.48 (dd, J = 10.1, 6.9 Hz, 1H, H4_B), 3.11 (app t, J = 9.8 Hz, 1H, H4_A); *trans*: δ (NH not observed), 8.57 (d, J = 4.9 Hz, 1H, H6'), 8.23 (d, J = 8.8 Hz, 2H, H3a'), 7.66 (app td, J = 7.7, 1.8 Hz, 1H, H4'), 7.55 (d, J = 8.8 Hz, 2H, H2a'), 7.31 (d, J = 7.7 Hz, 1H, H3'), 7.21 (dd, J = 7.7, 4.9 Hz, 1H, H5'), 5.83 (s, 1H, H2), 5.32 (s, 2H, H7), 4.66 (dd, J = 6.1, 4.5 Hz, 1H, H5), 3.38 – 3.43 (m, 1H, H4_B), 3.35 – 3.38 (m, 1H, H4_A); ¹³C NMR (CDCl₃, 101 MHz): *cis*: δ 170.5 (C6), 156.6 (C2'), 150.0 (C6'), 148.1 (C4a'), 142.5 (C1a'), 137.0 (C4'), 128.7 (C3a'), 124.1 (C2a'), 123.6 (C3'), 122.3 (C5'), 71.6 (C5), 66.3 (C2), 65.8 (C7), 39.5 (C4); *trans*: δ 171.5 (C6), 158.3 (C2'), 149.7 (C6'), 148.0 (C4a'), 142.7 (C1a'), 137.0 (C4'), 128.6 (C3a'), 124.0 (C2a'), 123.3 (C3'), 121.9 (C5'), 71.1 (C5), 65.8 (C2), 65.7 (C7), 38.7 (C4); m/z (ESI⁺) 346 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for C₁₆H₁₆O₄N₃S [M+H]⁺ 346.0856; found 346.0855.

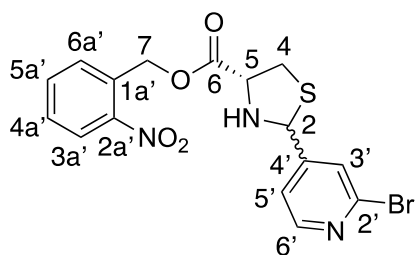
(5R)-2-(2-Bromopyridin-4-yl)-5-(4-nitrobenzyloxycarbonyl)-1,3-thiazolidine, 79b



Synthesised from **72** (1 eqv. 7.4 mmol) with 2-bromopyridine-4-carbaldehyde; yield (2.4 g, 77%); yellow oil; an inseparable 0.5 : 1 *cis* and *trans* diastereomers; $R_f = 0.41$ (petrol : EtOAc; 3 : 2);

$\nu_{\max}/\text{cm}^{-1}$ 3321 (br. s, N-H), 1739 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): minor *cis* isomer: δ (NH not observed), 8.23 (d, $J = 5.1$ Hz, 1H, H6'), 8.07 (d, $J = 8.9$ Hz, 2H, H3a'), 7.58 – 7.57 (m, 1H, H3'), 7.48 (d, $J = 8.9$ Hz, 2H, H2a'), 7.33 (d, $J = 5.1$ Hz, 1H, H5'), 5.45 (s, 1H, H2), 5.26 (s, 2H, H7), 4.13 – 4.05 (m, 1H, H5), 3.43 (dd, $J = 10.4, 7.0$ Hz, 1H, H4_B), 3.14 – 3.05 (m, 1H, H4_A); major *trans* isomer: δ (NH not observed), 8.18 (d, $J = 5.1$, 1H, H6'), 8.12 (d, $J = 8.9$ Hz, 2H, H3a'), 7.54 – 7.52 (m, 1H, H3'), 7.48 (d, $J = 8.9$ Hz, 2H, H2a'), 7.27 (d, $J = 5.1$ Hz, 1H, H5'), 5.71 (s, 1H, H2), 5.27 (s, 2H, H7), 4.13 – 4.05 (m, 1H, H5), 3.34 (dd, $J = 10.6, 6.8$ Hz, 1H, H4_B), 3.08 (dd, $J = 10.6, 6.6$ Hz, 1H, H4_A); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): minor *cis* isomer: δ 170.3 (C6), 150.7 (C6'), 150.1 (C4'), 147.6 (C4a'), 142.2, 142.1 (C1a' and C2'), 128.5 (C3a'), 126.8 (C3'), 123.3 (C2a'), 121.5 (C5'), 69.4 (C5), 65.2 (C7), 63.4 (C2), 38.4 (C4); major *trans* isomer: δ 170.8 (C6), 154.5 (C6'), 149.9 (C4'), 147.6 (C4a'), 142.2, 142.1 (C1a' and C2'), 128.4 (C3a'), 125.7 (C3'), 123.7 (C2a'), 121.0 (C5'), 67.8 (C5), 65.7 (C7), 64.0 (C2), 37.8 (C4); m/z (ESI⁺) 424 and 426 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for C₁₆H₁₅O₄N₃BrS [M+H]⁺ 423.9961 and 425.9940; found 423.9961 and 425.9938.

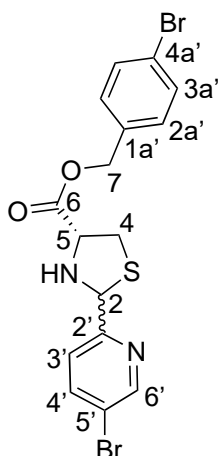
(5*R*)-2-(2-Bromopyridin-4-yl)-5-(2-nitrobenzyloxycarbonyl)-1,3-thiazolidine, 80b



Synthesised from **73** (1 eqv. 7.9 mmol) with 2-bromopyridine-4-carbaldehyde; yield (2.4 g, 72%); pale yellow oil; an inseparable 0.5 : 1 *cis* and *trans* diastereomers; $R_f = 0.61$ (petrol : EtOAc; 3 : 2); $\nu_{\max}/\text{cm}^{-1}$

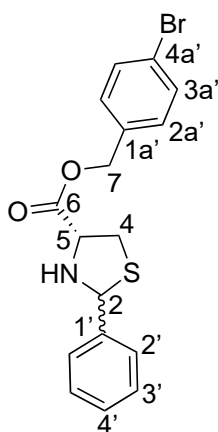
3291 (br. s, N-H), 1737 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ (NH not observed), 8.29 (d, $J = 5.1$ Hz, 1H, H6' *cis*), 8.24 (d, $J = 5.1$ Hz, 1H, H6' *trans*), 8.07 (d, $J = 8.2$ Hz, 2H, H3a' *cis* and *trans*), 7.68 – 7.48 (m, 8H, H4a', H5a', H6a' and H3', *cis* and *trans*), 7.35 (d, $J = 5.1$ Hz, 1H, H5' *cis*), 7.31 – 7.28 (m, 1H, H5' *trans*), 5.72 (s, 1H, H2 *trans*), 5.65 – 5.49 (m, 4H, H7 *cis* and *trans*), 5.45 (s, 1H, H2 *cis*), 4.12 – 4.04 (m, 2H, H5 *cis* and *trans*), 3.45 (dd, $J = 10.4, 7.0$ Hz, 1H, H4_B *cis*), 3.35 (dd, $J = 10.6, 6.8$ Hz, 1H, H4_B *trans*), 3.17 – 3.09 (m, 2H, H4_A *cis* and *trans*); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): minor *cis* isomer: δ 170.2 (C6), 150.6 (C6'), 150.4 (C4'), 147.7 (C2a'), 142.5 (C2'), 133.9 (C6a'), 130.7 (C1a'), 129.6 (C5a'), 129.5 (C4a'), 126.6 (C3'), 125.2 (C3a'), 121.5 (C5'), 69.6 (C5), 65.4 (C7), 64.2 (C2), 38.6 (C4); major *trans* isomer: δ 170.7 (C6), 154.6 (C6'), 150.1 (C4'), 147.7 (C2a'), 142.4 (C2'), 133.9 (C6a'), 130.8 (C1a'), 129.6 (C5a'), 129.4 (C4a'), 125.8 (C3'), 125.2 (C3a'), 121.2 (C5'), 67.9 (C5), 64.3 (C7), 64.2 (C2), 38.0 (C4); m/z (ESI⁺) 424 and 426 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for $\text{C}_{16}\text{H}_{15}\text{O}_4\text{N}_3\text{BrS}$ [M+H]⁺ 423.9961 and 425.9940; found 423.9962 and 425.9940.

(5R)-5-(4-Bromobenzyloxycarbonyl)-2-(5-bromopyridin-2-yl)-1,3-thiazolidine, 81c



Synthesised from **74** (1 eqv. 4.0 mmol) with 5-bromopyridine-2-carbaldehyde; yield (1.0 g, 55%); pale yellow solid, m.p. 125 – 130 °C; an inseparable mixture of 4.5 : 1 *cis* and *trans* diastereomers; $R_f = 0.21$ (petrol : EtOAc; 17 : 3); $\nu_{\max}/\text{cm}^{-1}$ 3290 (br. s, N-H), 1729 (s, C=O); ^1H NMR (CDCl_3 , 400 MHz): major *cis* isomer: δ 8.66 (d, $J = 2.1$ Hz, 1H, H6'), 7.79 (dd, $J = 8.2, 2.3$ Hz, 1H, H4'), 7.50 (d, $J = 8.4$ Hz, 2H, H3a'), 7.25 (app d, $J = 6.7$ Hz, 3H, H2a' and H3'), 5.60 (s, 1H, H2), 5.20 (s, 2H, H7), 4.08 – 3.99 (m, 1H, H5), 3.75 (br. s, 1H, NH), 3.42 (dd, $J = 10.1, 6.6$ Hz, 1H, H4_B), 3.05 (app t, $J = 9.8$ Hz, 1H, H4_A); minor *trans* isomer: δ (NH not observed), 8.60 (d, $J = 2.0$ Hz, 1H, H6'), 7.77 (dd, $J = 8.2, 2.3$ Hz, 1H, H4'), 7.50 (d, $J = 8.2$ Hz, 2H, H3a'), 7.19 (d, $J = 8.2$ Hz, 3H, H2a' and H3'), 5.80 (s, 1H, H2), 5.17 (s, 2H, H7), 4.51 (dd, $J = 6.8, 4.6$ Hz, 1H, H5), 3.35 (dd, $J = 10.7, 6.8$ Hz, 1H, H4_B), 3.27 (dd, $J = 10.7, 4.6$ Hz, 1H, H4_A); ^{13}C NMR (CDCl_3 , 101 MHz): major *cis* isomer: δ 170.5 (C6), 155.6 (C2'), 151.1 (C6'), 139.5 (C4'), 134.3 (C1a'), 132.0 (C3a'), 130.2 (C2a'), 123.3 (C3'), 122.9 (C4a'), 120.3 (C5'), 71.0 (C5), 66.6 (C7), 66.3 (C2), 39.5 (C4); minor *trans* isomer: δ 171.4 (C6), 155.6 (C2'), 150.8 (C6'), 139.5 (C4'), 134.5 (C1a'), 132.0 (C3a'), 130.1 (C2a'), 123.3 (C3'), 122.9 (C4a'), 119.8 (C5'), 70.5 (C5), 66.6 (C7), 65.7 (C2), 38.7 (C4); m/z (ESI⁺) 479 (50%), 480 ([M+Na]⁺, 100%) and 483 (50%); HRMS (ESI⁺) m/z calcd for $\text{C}_{16}\text{H}_{14}\text{O}_2\text{N}_2\text{Br}_2\text{NaS}$ [M+Na]⁺ 478.9035, 480.9014 and 482.8993; found 478.9036, 480.9013 and 482.8991.

(5R)-5-(4-Bromobenzyloxycarbonyl)-2-phenyl-1,3-thiazolidine, 81e

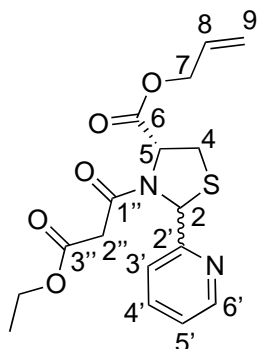


Synthesised from **74** (1 eqv. 4.4 mmol) with benzaldehyde; yield (1.5 g, 91%); pale yellow solid, m.p. 88 °C; an inseparable mixture of 1.7 : 1 *cis* and *trans* diastereomers; $R_f = 0.16$ (petrol : EtOAc; 9 : 1); $\nu_{\max}/\text{cm}^{-1}$ 3333 (br. s, N-H), 1733 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): major *cis* isomer: δ (NH not observed), 7.48 – 7.53 (m, 4H, H2' and H3a'), 7.30 – 7.39 (m, 3H, H4' and H3'), 7.24 (d, $J = 8.5$ Hz, 2H, H2a'), 5.55 (s, 1H, H2), 5.17 (s, 2H, H7), 3.96 – 4.05 (m, 1H, H5), 3.46 (dd, $J = 10.6, 7.1$ Hz, 1H, H4_B), 3.09 (dd, $J = 10.6, 8.9$ Hz, 1H, H4_A); minor *trans* isomer: δ (NH not observed), 7.45 – 7.53 (m, 4H, H2' and H3a'), 7.30 – 7.39 (m, 3H, H4' and H3'), 7.24 (d, $J = 8.5$ Hz, 2H, H2a'), 5.80 (s, 1H, H2), 5.16 (app d, $J = 1.5$ Hz, 2H, H7), 4.25 (dd, $J = 7.2, 5.7$ Hz, 1H, H5), 3.39 (dd, $J = 10.6, 7.2$ Hz, 1H, H4_B), 3.19 (dd, $J = 10.6, 5.7$ Hz, 1H, H4_A); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): major *cis* isomer: δ 171.1 (C6), 138.2 (C1'), 134.2 (C1a'), 132.0 (C3a'), 130.2 (C2a'), 128.8 (C3'), 128.6 (C4'), 127.5 (C2'), 122.9 (C4a'), 72.8 (C5), 66.7 (C7), 65.7 (C2), 39.3 (C4); minor *trans* isomer: δ 171.7 (C6), 141.2 (C1'), 134.4 (C1a'), 132.0 (C3a'), 130.1 (C2a'), 128.9 (C3'), 128.1 (C4'), 127.1 (C2'), 122.8 (C4a'), 71.0 (C5), 66.5 (C7), 64.5 (C2), 38.2 (C4); m/z (ESI⁺) 378 and 380 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for $\text{C}_{17}\text{H}_{17}\text{O}_2\text{NBrS}$ [M+H]⁺ 378.0158 and 380.0137; found 378.0159 and 380.0137.

N-Acylthiazolidines **82-88** were synthesised from the respective thiazolidines **75-81** by general procedure **B** (section 6.2.2).

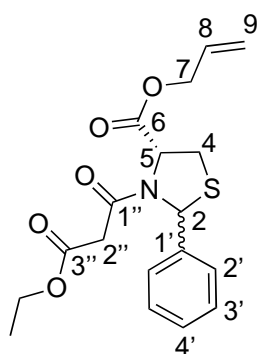
(5*R*)-5-Allyloxycarbonyl-1-(3-ethoxy-3-oxopropanoyl)-2-(pyridin-2-yl)-1,3-thiazolidine,

82a



Synthesised from **75a** (1 eqv. 6 mmol); yield (1.3 g, 58%); yellow oil; an inseparable mixture of 3.1 : 1 *cis* and *trans* diastereomers; $R_f = 0.24$ (petrol : EtOAc; 1 : 1); $\nu_{\max}/\text{cm}^{-1}$ 1735 (s, C=O), 1663 (s, C=O); ^1H NMR (CDCl_3 , 400 MHz): major *cis* isomer as a 1 : 0.2 mixture of rotamers; minor *trans* isomer as a 1 : 0.7 mixture of rotamers: δ 8.54 – 8.51 (m, 1H, H6'), 8.06 (d, $J = 7.9$ Hz, 1H, H3'), 7.71 (td, $J = 7.7, 1.8$ Hz, 1H, H4'), 7.22 – 7.18 (m, 1H, H5'), 6.43 (s, H2 *cis* minor rotamer), 6.20 (s, H2 *trans* minor rotamer), 6.16 (s, 1H, H2 *cis* major rotamer), 6.13 (s, H2 *trans* major rotamer), 5.95 – 5.84 (m, 1H, H8), 5.33 (dd, $J = 17.2, 1.4$ Hz, 2H, H9 *cis* major rotamer), 5.27 – 5.19 (m, H9 minor rotamer), 5.01 (dd, $J = 8.2, 6.7$ Hz, 1H, H5), 4.74 – 4.58 (m, 2H, H7), 4.19 – 3.98 (m, 2H, OCH_2CH_3), 3.60 – 3.00 (m, 4H, H4_B, H4_A, H2''_A and H2''_B), 1.28 – 1.11 (m, 3H, OCH_2CH_3); ^{13}C NMR (CDCl_3 , 101 MHz): major *cis* isomer, major rotamer: δ 169.6 (C6), 166.4 (C3''), 165.6 (C1''), 159.4 (C2'), 149.6 (C6'), 137.6 (C4'), 131.5 (C8), 123.3 (C3'), 120.7 (C5'), 118.9 (C9), 67.7 (C7), 66.4 (C5), 64.9 (C2), 61.7 (OCH_2CH_3), 42.1 (C2''), 31.8 (C4), 14.0 (OCH_2CH_3); m/z (ESI⁺) 365 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for $\text{C}_{17}\text{H}_{21}\text{O}_5\text{N}_2\text{S}$ [M+H]⁺ 365.1166; found 365.1167.

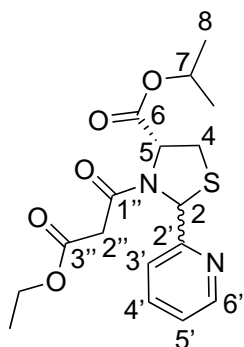
(5R)-5-Allyloxycarbonyl-1-(3-ethoxy-3-oxopropanoyl)-2-phenyl-1,3-thiazolidine, 82e



Synthesised from **75e** (1 eqv. 4.8 mmol); yield (1.3 g, 74%); yellow oil; an inseparable mixture of 1.2 : 1 *cis* and *trans* diastereomers; R_f = 0.26 (petrol : EtOAc; 4 : 1); $\nu_{\max}/\text{cm}^{-1}$ 1736 (s, C=O), 1661 (s, C=O); ^1H NMR (CDCl_3 , 400 MHz): major *cis* isomer as a 1 : 0.2 mixture of rotamers; minor *trans* isomer as a 1 : 0.3 mixture of rotamers: δ

7.66 (d, $J = 7.4$ Hz, 2H, H_2' *cis*), 7.52 (d, $J = 7.4$ Hz, H_2' minor rotamer), 7.38 (t, $J = 7.4$ Hz, 4H, H_3'), 7.33 (d, $J = 7.4$ Hz, 2H, H_2' *trans*), 7.22 (d, $J = 7.4$ Hz, 2H, H_4'), 6.37 (s, H_2 *cis* minor rotamer), 6.33 (s, H_2 *trans* minor rotamer), 6.19 (s, 1H, H_2 *trans* major rotamer), 6.15 (s, 1H, H_2 *cis* major rotamer), 5.86 – 6.04 (m, 2H, H_8 *cis* and *trans*), 5.19 – 5.44 (m, 5H, H_9 , H_5 *trans*), 5.10 (app t, $J = 6.8$ Hz, 1H, H_5 , *cis* major), 4.66 – 4.80 (m, 4H, H_7), 4.03 – 4.25 (m, 4H, OCH_2CH_3), 3.21 – 3.61 (m, 6H, $\text{H}_{4\text{B}}$, $\text{H}_{2''\text{A}}$, $\text{H}_{2''\text{B}}$), 3.11 (dd, $J = 15.3, 8.4$ Hz, 2H, $\text{H}_{4\text{A}}$), 1.19 – 1.35 (m, 6H, OCH_2CH_3); $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 126 MHz): δ 169.5, 168.8 (C_6 major and minor), 166.9, 166.4 (C_3'' major and minor), 165.7, 165.5 (C_1'' major, and minor), 142.2, 142.0, 139.9, 138.8 (C_1' major, minor and rotamers), 131.7, 131.6, 131.1 (C_8 major, minor and rotamers), 129.4, 129.2 (C_3' major and minor), 128.8, 128.7, 128.4, 128.1 (C_4' major, minor and rotamers), 127.2, 126.6, 125.1, 124.9 (C_2' major, minor and rotamers), 120.0, 119.8, 119.1, 118.8 (C_9 major, minor and rotamers), 66.5, 66.5 (C_7 major and minor), 65.2, 65.0 (C_5 major and minor), 64.5, 64.3, 64.1 (C_2 major, minor and rotamers), 61.9, 61.9, 61.8, 61.6 (OCH_2CH_3 major, minor and rotamers), 43.6, 43.1, 42.6, 42.3 (C_2'' major, minor and rotamers), 33.2, 32.6, 32.3, 31.2 (C_4 major, minor and rotamers), 14.3, 14.2, 14.2, 14.1 (OCH_2CH_3 major, minor and rotamers); m/z (ESI^+) 386 ($[\text{M}+\text{Na}]^+$, 100%); HRMS (ESI^+) m/z calcd for $\text{C}_{18}\text{H}_{21}\text{O}_5\text{NNaS}$ $[\text{M}+\text{Na}]^+$ 386.1033; found 386.1032.

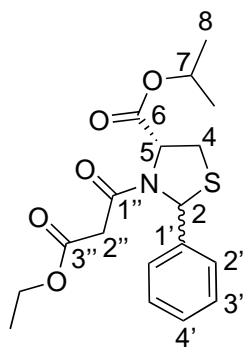
(5*R*)-1-(3-Ethoxy-3-oxopropanoyl)-5-isopropoxycarbonyl-2-(pyridin-2-yl)-1,3-thiazolidine, 83a



Synthesised from **76a** (1 eqv. 5.4 mmol); yield (1.4 g, 71%); orange oil; an inseparable mixture of 1.4 : 1 *cis* and *trans* diastereomers; $R_f = 0.32$ (petrol : EtOAc; 1 : 1); $\nu_{\max}/\text{cm}^{-1}$ 1737 (s, C=O), 1665 (s, C=O); ^1H NMR (CDCl_3 , 400 MHz): major *cis* isomer as a 1 : 0.2 mixture of rotamers; minor *trans* isomer as a 1 : 0.9 mixture of rotamers: δ 8.52 – 8.47 (m, 1H, H6' *cis*), 8.46 – 8.40 (m, 1H, H6' *trans*), 8.09 (d, $J = 8.0$ Hz, 1H, H3' *cis*), 7.70 (app td, $J = 8.0, 1.8$ Hz, 1H, H4' *cis*), 7.57 (app td, $J = 6.9, 6.1, 2.3$ Hz, 1H, H4' *trans*), 7.24 – 7.15 (m, 2H, H3' *trans* and H5' *cis*), 7.08 (dd, $J = 7.0, 5.4$ Hz, 1H, H5' *trans*), 6.40 (s, H2 *cis* minor rotamer), 6.17 (s, H2 *trans* minor rotamer), 6.14 (s, 1H, H2 *cis* major rotamer), 6.11 (s, 1H, H2 *trans* major rotamer), 5.24 – 4.98 (m, 3H, H7 *cis* and *trans* + H5), 4.89 (dd, $J = 8.6, 6.6$ Hz, 1H, H5), 4.17 – 3.97 (m, 4H, OCH_2CH_3 *cis* and *trans*), 3.59 – 2.95 (m, 8H, H4_B, H4_A, H2''_A and H2''_B, *cis* and *trans*), 1.29 – 1.08 (m, 18H, OCH_2CH_3 and H8, *cis* and *trans*); ^{13}C NMR (CDCl_3 , 101 MHz): δ 169.3, 169.2, 169.0, 168.5 (C6 major, minor and rotamers), 166.9, 166.8, 166.4, 166.2 (C3'' major, minor and rotamers), 165.4, 165.2, 165.0, 164.7 (C1'' major, minor and rotamers), 160.3, 160.3, 159.5, 158.5 (C2' major, minor and rotamers), 149.9, 149.5, 149.3, 148.9 (C6' major, minor and rotamers), 137.5, 137.4, 136.8, 136.6 (C4' major, minor and rotamers), 123.2, 123.1, 122.5, 122.5 (C3' major, minor and rotamers), 120.7, 120.3, 119.6, 119.4 (C5' major, minor and rotamers), 70.5, 70.4, 69.6, 69.3 (C7 major, minor and rotamers), 67.7, 67.6, 66.4, 66.0 (C5 major, minor and rotamers), 65.2, 64.2, 64.2, 64.1 (C2 major, minor and rotamers), 61.6, 61.5, 61.3 (OCH_2CH_3 major, minor and rotamers), 43.1, 42.6, 42.3, 42.0 (C2'' major, minor and rotamers), 33.8, 33.6, 31.7, 31.3 (C4 major, minor and rotamers), 21.7, 21.6 (C8 major,

minor and rotamers), 14.0, 14.0, 13.9 (OCH₂CH₃ major, minor and rotamers); *m/z* (ESI⁺) 367 ([M+H]⁺, 100%); HRMS (ESI⁺) *m/z* calcd for C₁₇H₂₃O₅N₂S [M+H]⁺ 367.1322; found 367.1323.

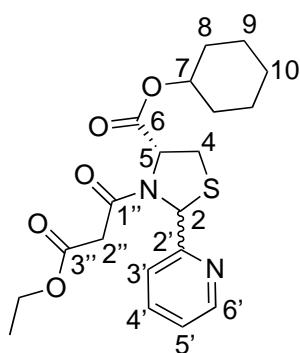
(5*R*)-1-(3-Ethoxy-3-oxopropanoyl)-5-isopropoxycarbonyl-2-phenyl-1,3-thiazolidine, 83e



Synthesised from **76e** (1 eqv. 4.0 mmol); yield (1.4 g, 97%); pale yellow oil; an inseparable mixture of 1 : 1 *cis* and *trans* diastereomers; *R_f* = 0.24 (petrol : EtOAc; 3 : 1); *v*_{max}/cm⁻¹ 1737 (s, C=O), 1663 (s, C=O); ¹H NMR (CDCl₃, 400 MHz): major *cis* isomer as a 1 : 0.2 mixture of rotamers; minor *trans* isomer as a 1 : 0.4 mixture of rotamers: δ 7.68 (d, *J* = 7.3 Hz, 2H, H2' *cis*), 7.53 (d, *J* = 7.4 Hz, H2' minor rotamers), 7.27 – 7.42 (m, 6H, H3' and H2' *trans*), 7.22 (d, *J* = 7.1 Hz, 2H, H4'), 6.37 (s, H2 *cis* minor rotamer), 6.32 (s, H2 *trans* minor rotamer), 6.17 (s, 1H, H2 *trans* major rotamer), 6.15 (s, 1H, H2 *cis* major rotamer), 5.26 (dd, *J* = 7.0, 1.2 Hz, 1H, H5 *trans*), 5.05 – 5.19 (m, 2H, H7), 4.99 (app t, *J* = 7.0 Hz, 1H, H5 *cis*), 4.11 – 4.16 (m, 2H, OCH₂CH₃ *trans*), 4.08 (q, *J* = 7.1 Hz, 2H, OCH₂CH₃ *cis*), 3.14 – 3.61 (m, 6H, H4_B, H2''_A, H2''_B), 3.10 (app dd, *J* = 15.3, 13.4 Hz, 2H, H4_A), 1.34 (d, *J* = 6.3 Hz, H8 *cis* and *trans* minor rotamers), 1.30 (app dd, *J* = 6.2, 2.1 Hz, 12H, H8 *cis* and *trans* major rotamers), 1.19 – 1.26 (m, 6H, OCH₂CH₃); ¹³C NMR (CDCl₃, 101 MHz): δ 169.3, 168.5 (C6 major and minor), 166.9, 166.5 (C3'' major and minor), 165.5, 165.4 (C1'' major, and minor), 140.3, 142.2, 142.2 (C1' major, minor and rotamers), 129.4, 129.1 (C3' major and minor), 128.7, 128.6, 128.3, 127.7 (C4' major, minor and rotamers), 127.2, 126.6, 125.1, 124.9 (C2' major, minor and rotamers), 70.8, 70.7, 69.7, 69.7 (C7 major, minor and rotamers), 66.5, 65.8, 65.3 (C5 major, minor and rotamers), 65.2, 64.6, 64.5, (C2 major, minor and rotamers), 62.0, 61.9, 61.7, 61.6 (OCH₂CH₃ major, minor and rotamers), 43.6,

43.0, 42.6, 42.3 (C2'' major, minor and rotamers), 34.0, 33.3, 32.2, 31.2 (C4 major, minor and rotamers), 21.9, 21.9, 21.8, 21.8 (C8 major, minor and rotamers), 14.2, 14.2, 14.1, 14.1 (OCH₂CH₃ major, minor and rotamers); *m/z* (ESI⁺) 366 ([M+H]⁺, 100%); HRMS (ESI⁺) *m/z* calcd for C₁₈H₂₄O₅NS [M+H]⁺ 366.1370; found 366.1371.

(5R)-5-Cyclohexyloxycarbonyl-1-(3-ethoxy-3-oxopropanoyl)-2-(pyridin-2-yl)-1,3-thiazolidine, 84a



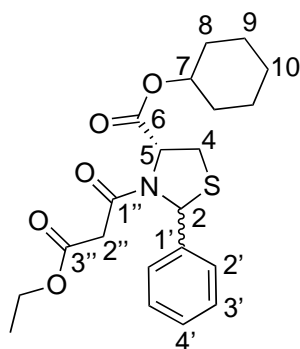
Synthesised from **77a** (1 eqv. 3.0 mmol); yield (0.8 g, 64%); orange-red oil; reaction from *cis-77a* produced a mixture of 4.8 : 1 *cis/trans* diastereomers, reaction from *trans-77a* produced a mixture of 0.2 : 1 *cis/trans* diastereomers; *R_f* = 0.37 (petrol : EtOAc; 1 : 1); $[\alpha]_D^{25} = -52.4$ (*c* = 1.13, CHCl₃), *cis*; $[\alpha]_D^{25} = -67.1$ (*c* =

1.65, CHCl₃), *trans*; $\nu_{\max}/\text{cm}^{-1}$ 1737 (s, C=O), 1664 (s, C=O); ¹H NMR (CDCl₃, 400 MHz): *cis* isomer as a 1 : 0.3 mixture of rotamers: δ 8.51 – 8.48 (m, 1H, H6'), 8.09 (d, *J* = 7.9 Hz, 1H, H3'), 7.69 (app td, *J* = 7.9, 1.7 Hz, 1H, H4'), 7.17 (dd, *J* = 7.9, 4.8 Hz, 1H, H5'), 6.39 (s, H2 minor rotamer), 6.14 (s, 1H, H2 major rotamer), 4.90 (dd, *J* = 8.6, 6.6 Hz, 1H, H5), 4.86 – 4.75 (m, 1H, H7), 4.16 – 3.96 (m, 2H, OCH₂CH₃), 3.31 (d, *J* = 15.6 Hz, 1H, H2''_B), 3.26 (dd, *J* = 12.0, 6.6 Hz, 1H, H4_B), 3.15 (dd, *J* = 12.0, 8.6 Hz, 1H, H4_A), 3.07 (d, *J* = 15.6 Hz, 1H, H2''_A), 1.91 – 1.75 (m, 2H, H8), 1.73 – 1.57 (m, 2H, H8), 1.54 – 1.19 (m, 6H, H9 and H10), 1.15 (t, *J* = 7.1 Hz, 3H, OCH₂CH₃); *trans* isomer as a 1 : 1 mixture of rotamers: δ 8.48 (d, *J* = 5.5 Hz, 1H, H6'), 8.42 (d, *J* = 4.3 Hz, 1H, H6'), 7.65 (app td, *J* = 7.8, 1.7 Hz, 1H, H4'), 7.55 (app td, *J* = 7.8, 1.8 Hz, 1H, H4'), 7.22 (d, *J* = 7.8 Hz, 1H, H3'), 7.18 (app d, *J* = 7.5 Hz, 2H, H3' and H5'), 7.07 (dd, *J* = 7.8, 4.3 Hz, 1H, H5'), 6.17 (s, 1H, H2), 6.10 (s, 1H, H2), 5.22 (app d, *J* = 7.0 Hz, 1H, H5), 5.06 (app d, *J* = 6.0 Hz, 1H, H5), 4.93 – 4.73 (m, 2H, H7), 4.15 – 4.06 (m, 2H,

OCH₂CH₃), 3.99 (q, $J = 7.1$ Hz, 2H, OCH₂CH₃), 3.73 (dd, $J = 12.0, 6.1$ Hz, 1H, H_{4B}), 3.52 (dd, $J = 12.0, 7.1$ Hz, 1H, H_{4B}), 3.38 (d, $J = 12.3$ Hz, 1H, H_{2''B}), 3.36 – 3.28 (m, 2H, H_{4A}), 3.25 (d, $J = 15.2$ Hz, 1H, H_{2''B}), 3.11 (d, $J = 12.3$ Hz, 1H, H_{2''A}), 2.98 (d, $J = 15.2$ Hz, 1H, H_{2''A}), 1.78 (br. s, 4H, H₈), 1.66 (br. s, 4H, H₈), 1.51 – 1.22 (m, 12H, H₉ and H₁₀), 1.18 (t, $J = 7.1$ Hz, 3H, OCH₂CH₃), 1.12 (t, $J = 7.1$ Hz, 3H, OCH₂CH₃); ¹³C NMR (CDCl₃, 101 MHz): *cis*, major rotamer: δ 169.2 (C₆), 166.3 (C_{3''}), 165.4 (C_{1''}), 159.5 (C_{2'}), 149.5 (C_{6'}), 137.4 (C_{4'}), 123.1 (C_{3'}), 120.6 (C_{5'}), 74.4 (C₇), 67.6 (C₅), 65.2 (C₂), 61.5 (OCH₂CH₃), 41.9 (C_{2''}), 33.9 (C₄), 31.7, 31.2 (C₈), 25.2 (C₁₀), 23.5 (C₉), 13.9 (OCH₂CH₃); *trans*, 2 rotamers: δ 169.1, 168.5 (C₆), 166.9, 166.2 (C_{3''}), 165.0, 164.7 (C_{1''}), 160.4, 160.3 (C_{2'}), 149.8, 149.3 (C_{6'}), 137.3, 136.7 (C_{4'}), 123.1, 122.4 (C_{3'}), 119.4, 119.4 (C_{5'}), 75.0, 73.9 (C₇), 66.5, 66.0 (C₅), 64.3, 64.2 (C₂), 61.5, 61.3 (OCH₂CH₃), 43.1, 42.3 (C_{2''}), 33.6 (C₄), 31.2, 31.2 (C₈), 25.2, 25.1 (C₁₀), 23.3, 23.3 (C₉), 14.0, 13.9 (OCH₂CH₃); m/z (ESI⁺) 407 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for C₂₀H₂₇O₅N₂S [M+H]⁺ 407.1635; found 407.1634.

(5*R*)-5-Cyclohexyloxycarbonyl-1-(3-ethoxy-3-oxopropanoyl)-2-phenyl-1,3-thiazolidine,

84e

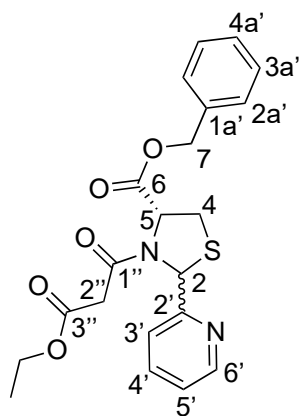


Synthesised from **77e** (1 eqv. 4 mmol); yield (1.6 g, 99%); pale yellow oil; inseparable mixture of 1.3 : 1 *cis* and *trans* diastereomers; $R_f = 0.28$ (petrol : EtOAc; 4 : 1); $\nu_{\max}/\text{cm}^{-1}$ 1736 (s, C=O), 1663 (s, C=O); ¹H NMR (CDCl₃, 400 MHz): major *cis* isomer as a 1 : 0.2 mixture of rotamers; minor *trans* isomer as a 1 : 0.4

mixture of rotamers: δ 7.68 (d, $J = 7.3$ Hz, 2H, H_{2'} *cis*), 7.53 (d, $J = 7.2$ Hz, H_{2'} minor rotamers), 7.26 – 7.42 (m, 6H, H_{3'} and H_{2'} *trans*), 7.22 (d, $J = 7.0$ Hz, 2H, H_{4'}), 6.37 (s, H₂ *trans* minor rotamer), 6.32 (s, H₂ *trans* major rotamer), 6.17 (s, 1H, H₂ *trans* major

rotamer), 6.15 (s, 1H, H2 *cis* major rotamer), 5.28 (dd, $J = 6.8, 0.9$ Hz, 1H, H5 *trans*), 5.14 (app d, $J = 5.4$ Hz, H5 minor rotamer), 5.01 (app t, $J = 7.0$ Hz, 1H, H5 *cis*), 4.83 – 4.95 (m, 2H, H7), 4.11 – 4.16 (m, 2H, OCH₂CH₃ *trans*), 4.08 (q, $J = 7.1$ Hz, 2H, OCH₂CH₃ *cis*), 3.25 (s, 2H, H2''_B *cis* and *trans*), 3.17 – 3.59 (m, 4H, H2''_A, H4_B), 3.10 (dd, $J = 15.3, 13.1$ Hz, 2H, H4_A), 1.95 (br. s, H8 minor rotamers), 1.86 (br. s, 4H, H8 *trans* major rotamer), 1.73 (br. s, 4H, H8 *cis* major rotamer), 1.34 – 1.59 (m, 12H, H9 and H10), 1.26 – 1.31 (m, OCH₂CH₃ minor rotamers), 1.22 – 1.26 (m, 3H, OCH₂CH₃ *cis* major rotamer), 1.18 – 1.22 (m, 3H, OCH₂CH₃ *trans* major rotamer); ¹³C{¹H} NMR (CDCl₃, 126 MHz): δ 169.3, 169.2, 168.5 (C6 major, minor and rotamers), 167.4, 167.3, 167.0, 166.5 (C3'' major, minor and rotamers), 165.5, 165.5, 165.3, 164.7 (C1'' major, minor and rotamers), 142.3, 142.2, 140.2, 139.0 (C1' major, minor and rotamers), 129.4, 129.1 (C3' major and minor), 128.7, 128.6, 128.3, 128.1 (C4' major, minor and rotamers), 127.2, 126.6, 125.1, 124.9 (C2' major, minor and rotamers), 75.5, 75.4, 74.6, 74.4 (C7 major, minor and rotamers), 67.4, 66.5, 65.8, 65.4 (C5 major, minor and rotamers), 65.2, 64.7, 64.6, 64.3 (C2 major, minor and rotamers), 62.1, 61.9, 61.7, 61.6 (OCH₂CH₃ major, minor and rotamers), 43.6, 43.0, 42.6, 42.3 (C2'' major, minor and rotamers), 34.1, 33.4, 32.3, 31.6 (C4 major, minor and rotamers), 31.6, 31.5, 31.5, 31.3 (C8 major, minor and rotamers), 25.5, 25.5, 25.3, 25.3 (C10 major, minor and rotamers), 23.8, 23.7, 23.6 (C9 major, minor and rotamers), 14.2, 14.2, 14.2 (OCH₂CH₃ major, minor and rotamers); m/z (ESI⁺) 428 ([M+Na]⁺, 100%); HRMS (ESI⁺) m/z calcd for C₂₁H₂₇O₅NNaS [M+Na]⁺ 428.1502; found 428.1497.

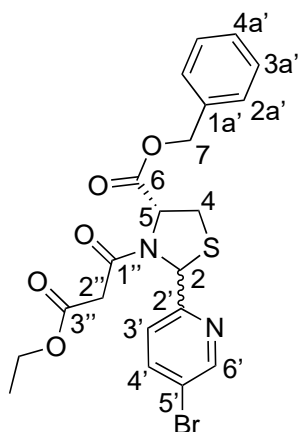
(5R)-5-Benzyloxycarbonyl-1-(3-ethoxy-3-oxopropanoyl)-2-phenyl-1,3-thiazolidine, 85a



Synthesised from **78a** (1 eqv. 4.2 mmol); yield (0.85 g, 49%); pale yellow oil; an inseparable 1.7 : 1 *cis* and *trans* diastereomers; $R_f = 0.23$ (petrol : EtOAc; 1 : 1); $\nu_{\max}/\text{cm}^{-1}$ 1740 (s, C=O), 1664 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): major *cis* isomer as a 1 : 0.2 mixture of rotamers; minor *trans* isomer as a 1 : 0.7 mixture of rotamers: δ 8.58 – 8.54 (m, 2H, H6' *cis* and *trans*), 8.03 (d, $J = 7.9$ Hz, 2H, H3' *cis* and *trans*), 7.63 (app td, $J = 7.9, 1.9$ Hz, 2H, H4' *cis* and *trans*), 7.42 – 7.30 (m, 10H, H2a', H3a' and H4a', *cis* and *trans*), 7.25 – 7.20 (m, 2H, H5' *cis* and *trans*), 6.48 (s, H2 *cis* minor rotamer), 6.27 (s, H2 *trans* minor rotamer), 6.22 (s, 1H, H2 *cis* major rotamer), 6.19 (s, 1H, H2 *trans* major rotamer), 5.30 – 5.22 (m, 5H, H7 *cis* and *trans* and H5 *trans*), 5.10 (dd, $J = 8.2, 6.7$ Hz, 1H, H5 *cis*), 4.22 – 4.04 (m, 4H, OCH_2CH_3 *cis* and *trans*), 3.65 – 3.08 (m, 8H H2''_A, H2''_B, H4_A and H4_B, *cis* and *trans*), 1.30 – 1.16 (m, 6H, OCH_2CH_3 *cis* and *trans*); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ 169.7, 169.6, 169.5, 169.0 (C6 major, minor and rotamers), 166.9, 166.9, 166.4, 166.2 (C3'' major, minor and rotamers), 165.6, 165.2, 165.0, 164.7 (C1'' major, minor and rotamers), 160.2, 160.1, 159.2, 158.4 (C2' major, minor and rotamers), 149.9, 149.5, 149.2, 148.9 (C6' major, minor and rotamers), 137.5, 137.4, 136.9, 136.7 (C4' major, minor and rotamers), 135.4, 135.1, 134.8, 134.7 (C1a' major, minor and rotamers), 128.7, 128.7, 128.6, 128.5, 128.4, 128.4, 128.2, 128.0 (C2a', C3a' and C4a', major, minor and rotamers), 123.2, 123.2, 122.6 (C3' major, minor and rotamers), 120.6, 120.4, 119.7, 119.5 (C5' major, minor and rotamers), 68.1, 67.6, 67.5, 67.4 (C7 major, minor and rotamers), 66.4, 66.0 (C5 *cis* and *trans*), 65.0, 64.2, 64.0 (C2 major, minor and rotamers), 61.7, 61.6, 61.5, 61.4 (OCH_2CH_3 major, minor and rotamers), 43.1, 42.7, 42.3, 42.0 (C2'' major, minor and rotamers), 33.9, 33.5, 31.7, 31.3 (C4 major,

minor and rotamers), 14.0 (OCH₂CH₃ major, minor and rotamers); *m/z* (ESI⁺) 415 ([M+H]⁺, 100%); HRMS (ESI⁺) *m/z* calcd for C₂₁H₂₃O₅N₂S [M+H]⁺ 415.1322; found 415.1321.

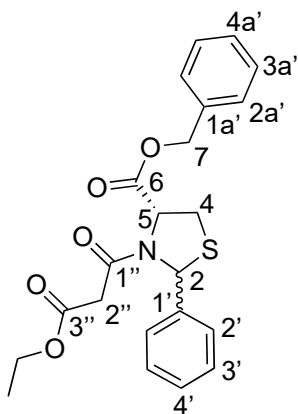
(5*R*)-5-(Benzyloxycarbonyl)-2-(5-bromopyridin-2-yl)-1-(3-ethoxy-3-oxopropanoyl)-1,3-thiazolidine, 85c



Synthesised from **78c** (1 eqv. 4.8 mmol); yield (1.6 g, 68%); pale yellow oil; an inseparable mixture of 1 : 1 *cis* and *trans* diastereomers; *R_f* = 0.29 (petrol : EtOAc; 3 : 1); *v*_{max}/cm⁻¹ 1736 (s, C=O), 1663 (s, C=O); ¹H NMR (CDCl₃, 400 MHz): major *cis* isomer as a 1 : 0.3 mixture of rotamers; minor *trans* isomer as a 1 : 0.9 mixture of rotamers: δ 8.60 (d, *J* = 2.2 Hz, H_{6'} *trans* minor rotamer), 8.58 (d, *J* = 2.2 Hz, 1H, H_{6'} *cis* rotamer), 8.53 (d, *J* = 2.2 Hz, 1H, H_{6'} *trans* major rotamer), 7.94 (d, *J* = 8.4 Hz, 1H, H_{3'} *cis*), 7.83 (dd, *J* = 8.3, 2.3 Hz, H_{4'} *trans* minor rotamer), 7.73 (app td, *J* = 8.4, 2.3 Hz, 2H, H_{4'} *cis* and *trans* major rotamer), 7.37 (br. s, 10H, H_{2a'}, H_{3a'} and H_{4a'}, *cis*, *trans* and rotamers), 7.20 (d, *J* = 8.3 Hz, 1H, H_{3'} *trans* major rotamer), 7.16 (d, *J* = 8.3 Hz, H_{3'} *trans* minor rotamer), 6.39 (s, H₂ *cis* minor rotamer), 6.18 (s, 1H, H₂ *trans* major rotamer), 6.15 (s, 1H, H₂ *cis* major rotamer), 6.13 (s, H₂ *trans* minor rotamer), 5.38 – 5.06 (m, 6H, H₇ and H₅, *cis*, *trans* and rotamers), 4.25 – 4.00 (m, 4H, OCH₂CH₃, *cis*, *trans* and rotamers), 3.82 (dd, *J* = 11.9, 6.2 Hz, H_{4_B} *trans* minor rotamer), 3.56 (dd, *J* = 12.4, 7.1 Hz, 1H, H_{4_B} *trans* major rotamer), 3.44 – 3.05 (m, 7H, H_{2''_B}, H_{2''_A} and H_{4_A}, *cis*, *trans* and rotamers + H_{4_B} *cis*), 1.29 – 1.16 (m, 6H, OCH₂CH₃, *cis*, *trans* and rotamers); ¹³C NMR (CDCl₃, 101 MHz): δ 169.8, 169.7, 169.0 (C₆ major, minor and rotamers), 167.0, 166.4, 166.2 (C_{3''} major, minor and rotamers), 165.6, 165.0, 164.8 (C_{1''} major, minor and rotamers), 159.2, 158.9, 158.0 (C_{2'} major, minor and rotamers), 151.1,

150.8, 150.7, 150.2 (C6' major, minor and rotamers), 140.2, 140.1, 139.4, 139.3 (C4' major, minor and rotamers), 135.6, 135.2, 134.9 (C1a' major, minor and rotamers), 128.9, 128.8, 128.7, 128.7, 128.5, 128.2 (C2a', C3a' and C4a' major, minor and rotamers), 122.4, 121.3, 121.0 (C3' major, minor and rotamers), 120.4, 119.4 (C5' major *cis* and minor *trans*), 68.4, 67.8, 67.7, 67.3 (C7 major, minor and rotamers), 66.1, 65.6, 65.1 (C5 major, minor and rotamers), 64.3, 64.2 (C2 major *cis* and minor *trans*), 62.0, 61.9, 61.8, 61.7 (OCH₂CH₃ major, minor and rotamers), 43.2, 42.6, 42.2, 41.7 (C2'' major, minor and rotamers), 34.1, 33.8, 32.0, 31.6 (C4 major, minor and rotamers), 14.3, 14.2 (OCH₂CH₃ major *cis* and minor *trans*); *m/z* (ESI⁺) 515 and 517 ([M+Na]⁺, 100%); HRMS (ESI⁺) *m/z* calcd for C₂₁H₂₁O₅N₂BrNaS [M+Na]⁺ 515.0247 and 517.0227; found 515.0249 and 517.0225.

(5R)-5-Benzyloxycarbonyl-1-(3-ethoxy-3-oxopropanoyl)-2-phenyl-1,3-thiazolidine, 85e

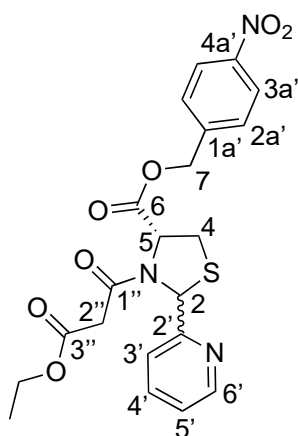


Synthesised from **78e** (1 eqv. 4.4 mmol); yield (1.8 g, 99%); pale greenish yellow oil; an inseparable 1.9 : 1 *cis* and *trans* diastereomers; *R_f* = 0.21 (petrol : EtOAc; 4 : 1); *v*_{max}/cm⁻¹ 1739 (s, C=O), 1662 (s, C=O); ¹H NMR (CDCl₃, 400 MHz): major *cis* isomer as a 1 : 0.1 mixture of rotamers; minor *trans* isomer as a 1 : 0.3 mixture of rotamers: δ 8.09 – 8.12 (m, H2' minor rotamers), 7.56

– 7.65 (m, 2H, H2' *cis* major rotamer), 7.48 (d, *J* = 7.9 Hz, 2H, H2' *trans* major rotamer), 7.28 – 7.41 (m, 14H, H4a', H3', H3a', H2a'), 7.21 (d, *J* = 8.0 Hz, 2H, H4'), 6.36 (s, H2 *cis* minor rotamer), 6.34 (s, H2 *trans* minor rotamer), 6.18 (s, 1H, H2 *trans* major rotamer), 6.14 (s, 1H, H2 *cis* major rotamer), 5.37 (app d, *J* = 6.8 Hz, 1H, H5 *trans*), 5.21 – 5.32 (m, 4H, H7), 5.12 (app t, *J* = 6.8 Hz, 1H, H5 *cis*), 4.10 – 4.21 (q, *J* = 7.1 Hz, 2H, OCH₂CH₃ *cis*),

4.04 (q, $J = 7.1$ Hz, 2H, OCH_2CH_3 *trans*), 3.18 – 3.56 (m, 6H, $\text{H}2''_{\text{A}}$, $\text{H}2''_{\text{B}}$, $\text{H}4_{\text{B}}$), 3.11 (dd, $J = 15.3$, 4.7 Hz, 2H, $\text{H}4_{\text{A}}$), 1.24 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3 *cis*), 1.19 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3 *trans*); ^{13}C NMR (CDCl_3 , 101 MHz): δ 169.6, 169.0 ($\text{C}6$ major and minor), 166.9, 166.4 ($\text{C}3''$ major and minor), 165.7, 165.6 ($\text{C}1''$ major and minor), 142.0, 139.8 ($\text{C}1'$ major and minor), 135.5, 135.3 ($\text{C}1\text{a}'$ major and minor), 130.2, 129.4, 129.1 ($\text{C}3'$), 128.9, 128.8, 128.7, 128.7 ($\text{C}4\text{a}'$ and $\text{C}3\text{a}'$), 128.6, 128.6, 128.5, 128.2 ($\text{C}4'$ and $\text{C}2\text{a}'$), 127.2, 126.6, 125.1, 124.9 ($\text{C}2'$ major, minor and rotamers), 67.7, 67.7 ($\text{C}7$ major and minor), 66.5 ($\text{C}5$ major and minor), 65.2, 65.1, 64.3 ($\text{C}2$ major, minor and rotamers), 61.9, 61.7, 61.6 (OCH_2CH_3 major, minor and rotamers), 42.6, 42.3 ($\text{C}2''$ major and minor), 32.2, 31.2 ($\text{C}4$ major and minor), 14.2, 14.1 (OCH_2CH_3 major and minor); m/z (ESI^+) 436 ($[\text{M}+\text{Na}]^+$, 100%); HRMS (ESI^+) m/z calcd for $\text{C}_{22}\text{H}_{23}\text{O}_5\text{NNaS}$ $[\text{M}+\text{Na}]^+$ 436.1200; found 436.1191.

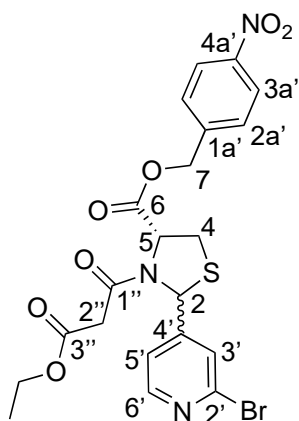
(5*R*)-1-(3-Ethoxy-3-oxopropanoyl)-5-(4-nitrobenzyloxycarbonyl)-2-(pyridin-2-yl)-1,3-thiazolidine, 86a



Synthesised from **79a** (1 eqv. 2.4 mmol); yield (1.1 g, 99%); pale yellow oil; inseparable 8.8 : 1 *cis* and *trans* diastereomers; $R_f = 0.22$ (petrol : EtOAc; 1 : 1); $\nu_{\text{max}}/\text{cm}^{-1}$ 1741 (s, C=O), 1662 (s, C=O); ^1H NMR (CDCl_3 , 400 MHz): major *cis* isomer as a 1 : 0.1 mixture of rotamers; minor *trans* isomer as a 1 : 0.4 mixture of rotamers: δ 8.54 (app dd, $J = 5.2$, 2.1 Hz, 1H, $\text{H}6'$), 8.20 (d, $J = 8.8$ Hz, 2H, $\text{H}3\text{a}'$), 7.98 (d, $J = 7.9$ Hz, 1H, $\text{H}3'$), 7.68 (app td, $J = 7.9$, 1.8 Hz, 1H, $\text{H}4'$), 7.54 (d, $J = 8.8$ Hz, 2H, $\text{H}2\text{a}'$), 7.22 (app ddd, $J = 7.9$, 5.2, 1.0 Hz, 1H, $\text{H}5'$), 6.47 (s, $\text{H}2$ *cis* minor rotamer), 6.24 (s, $\text{H}2$ *trans* minor rotamer), 6.20 (s, 1H, $\text{H}2$ *cis* major rotamer), 6.17 (s, $\text{H}2$ *trans* major rotamer), 5.38 (d, $J = 13.5$ Hz, 1H, $\text{H}7_{\text{B}}$), 5.27 (d, $J = 13.5$ Hz, 1H, $\text{H}7_{\text{A}}$), 5.09 (dd, $J = 8.5$, 6.6

Hz, 1H, H5), 4.15 – 4.07 (m, 2H, OCH₂CH₃), 3.40 (d, *J* = 15.7 Hz, 1H, H2''_B), 3.35 (dd, *J* = 12.0, 6.6 Hz, 1H, H4_B), 3.26 (dd, *J* = 12.0, 8.5 Hz, 1H, H4_A), 3.17 (d, *J* = 15.7 Hz, 1H, H2''_A), 1.21 (t, *J* = 7.2 Hz, 3H, OCH₂CH₃); ¹³C NMR (CDCl₃, 101 MHz): major *cis* isomer, major rotamer: δ 169.7 (C6), 166.4 (C3''), 165.8 (C1''), 159.2 (C2'), 149.7 (C6'), 147.8 (C4a'), 142.5 (C1a'), 137.6 (C4'), 128.4 (C3a'), 123.9 (C2a'), 123.4 (C3'), 120.6 (C5'), 67.6 (C7), 65.9 (C5), 64.9 (C2), 61.8 (OCH₂CH₃), 42.1 (C2''), 31.8 (C4), 14.1 (OCH₂CH₃); *m/z* (ESI⁺) 460 ([M+H]⁺, 100%); HRMS (ESI⁺) *m/z* calcd for C₂₁H₂₂O₇N₃S [M+H]⁺ 460.1173; found 460.1173.

(5*R*)-2-(2-bromopyridin-4-yl)1-(3-ethoxy-3-oxopropanoyl)-5-(4-nitrobenzyloxycarbonyl)-1,3-thiazolidine, 86b

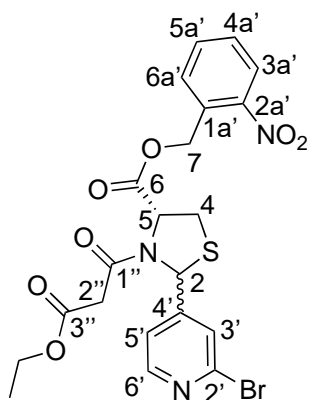


Synthesised from **79b** (1 eqv. 5.5 mmol); yield (2.1 g, 71%); pale greenish yellow oil; inseparable 1.5 : 1 *cis* and *trans* diastereomers; *R_f* = 0.24 (petrol : EtOAc; 3 : 2); *v*_{max}/cm⁻¹ 1738 (s, C=O), 1663 (s, C=O); ¹H NMR (CDCl₃, 400 MHz): major *cis* isomer as a 1 : 0.5 mixture of rotamers; minor *trans* isomer as a 1 : 0.8 mixture of rotamers: δ 8.40 – 8.27 (m, 2H, H6' *cis* and *trans*),

8.22 (d, *J* = 8.3 Hz, 4H, H3a' *cis* and *trans*), 7.83 (s, 1H, H3' *cis*), 7.62 – 7.47 (m, 4H, H2a' *cis* and *trans*), 7.39 – 7.32 (m, 2H, H5' *cis* and *trans*), 7.19 – 7.10 (m, 1H, H3' *trans*), 6.20 (s, H2 *cis* minor rotamer), 6.15 (s, H2 *trans* minor rotamer), 6.14 (s, 1H, H2 *cis* major rotamer), 6.13 (s, 1H, H2 *trans* major rotamer), 5.46 – 4.99 (m, 6H, H7 and H5), 4.28 – 4.02 (m, 4H, OCH₂CH₃), 3.56 – 3.13 (m, 8H, H2''_B H2''_A H4_B and H4_A), 1.32 – 1.16 (m, 6H, OCH₂CH₃); ¹³C NMR (CDCl₃, 101 MHz): δ 169.3, 169.2 (C6 major and minor), 167.1, 166.3 (C3'' major and minor), 165.2, 165.0 (C1'' major and minor), 153.7, 153.3, 152.3 (C6' major, minor and rotamers), 151.1, 150.8, 150.4, 150.2 (C4' major, minor and rotamers),

148.0 (C4a'), 143.3, 143.1, 142.7, 142.3, 141.7, 141.5 (C1a' and C2', major, minor and rotamers), 129.0, 128.8, 128.5, 128.4 (C3a' major, minor and rotamers), 126.0, 125.4 (C3' major and minor), 124.1, 124.0 (C2a' major and minor), 121.1, 120.3, 119.4, 119.1 (C5' major, minor and rotamers), 67.0, 66.9, 66.2 (C7 major, minor and rotamers), 65.4, 65.0, 64.3, 64.3 (C5 major, minor and rotamers), 64.2, 64.0, 63.8, 63.3 (C2 major, minor and rotamers), 62.3, 62.2, 62.0, 60.5 (OCH₂CH₃ major, minor and rotamers), 43.3, 43.0, 42.5, 42.2 (C2'' major, minor and rotamers), 34.5, 33.3, 32.0, 31.3 (C4 major, minor and rotamers), 14.3, 14.2, 14.1 (OCH₂CH₃ major, minor and rotamers); *m/z* (ESI⁺) 560 and 562 ([M+Na]⁺, 100%); HRMS (ESI⁺) *m/z* calcd for C₂₁H₂₀O₇N₃BrNaS [M+Na]⁺ 560.0098 and 562.0078; found 560.0098 and 562.0074.

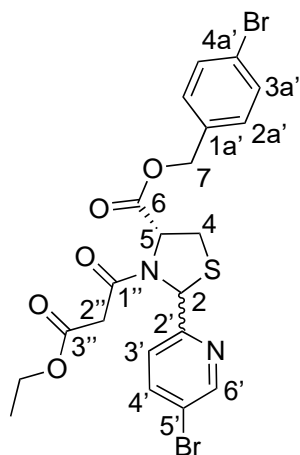
(5*R*)-2-(2-bromopyridin-4-yl)1-(3-ethoxy-3-oxopropanoyl)-5-(2-nitrobenzyloxycarbonyl)-1,3-thiazolidine, 87b



Synthesised from **80b** (1 eqv. 5.5 mmol); Yield (2.6 g, 88%); pale greenish yellow oil; inseparable 1.1 : 1 *cis* and *trans* diastereomers; *R_f* = 0.26 (petrol : EtOAc; 3 : 2); *v*_{max}/cm⁻¹ 1738 (s, C=O), 1663 (s, C=O); ¹H NMR (CDCl₃, 400 MHz): major *cis* isomer as a 1 : 0.6 mixture of rotamers; minor *trans* isomer as a 1 : 0.5 mixture of rotamers: δ 8.38 (d, *J* = 5.1 Hz, H6' *cis* minor rotamer), 8.35 (d, *J* = 5.1 Hz, 1H, H6' *cis* major rotamer), 8.28 (d, *J* = 5.2 Hz, 1H, H6' *trans* major rotamer), 8.19 (d, *J* = 5.1 Hz, H6' *trans* minor rotamer), 8.15 – 8.06 (m, 2H, H3a' *cis* and *trans*), 7.81 (s, 1H, H3' *cis*), 7.73 – 7.49 (m, 6H, H4a', H5a' and H6a', *cis* and *trans*), 7.39 (br. s, 1H, H5' *trans*), 7.34 (br. s, 1H, H5' *cis*), 7.18 (dd, *J* = 5.2, 1.4 Hz, 1H, H3' *trans* major rotamer), 7.13 (dd, *J* = 5.1, 1.4 Hz, H3' *trans* minor rotamer), 6.20 (s, H2 *cis* minor

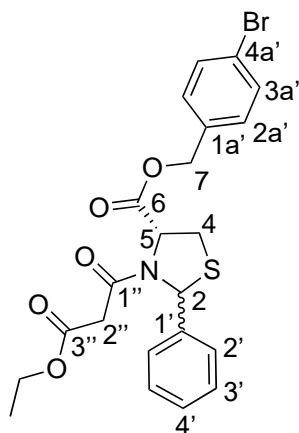
rotamer), 6.15 (s, 1H, H2 *cis* major rotamer), 6.14 (s, 1H, H2 *trans* major rotamer), 5.73 – 5.54 (m, 4H, H7, *cis* and *trans*), 5.36 (app d, $J = 6.5$ Hz, H5 *trans* minor rotamer), 5.28 (dd, $J = 5.2, 1.8$ Hz, 1H, H5 *trans* major rotamer), 5.18 – 5.11 (m, H5 *cis* minor rotamer), 5.03 (dd, $J = 9.2, 6.6$ Hz, 1H, H5 *cis* major rotamer), 4.31 – 3.99 (m, 4H, OCH₂CH₃ *cis* and *trans*), 3.58 – 3.11 (m, 8H, H2''_B H2''_A H4_B and H4_A, *cis* and *trans*), 1.34 – 1.14 (m, 6H, OCH₂CH₃ *cis* and *trans*); ¹³C NMR (CDCl₃, 101 MHz): δ 169.2, 169.1 (C6 major and minor), 167.3, 166.4 (C3'' major and minor), 165.2, 165.0 (C1'' major and minor), 153.8, 153.5, 152.3 (C6' major, minor and rotamers), 151.1, 150.9, 150.4, 150.2 (C4' major, minor and rotamers), 148.0 (C2a'), 143.8, 143.3, 143.1, 142.7 (C2' major, minor and rotamers), 134.2, 134.1 (C6a' major and minor), 131.3, 131.2, 130.4, 130.3 (C1a' major, minor and rotamers), 130.2, 130.0, 129.9 (C5a' major, minor and rotamers), 129.7, 129.6, 129.3, 129.2 (C4a' major, minor and rotamers), 126.1, 125.5, 125.3, 124.1 (C3' and C3a', major, minor and rotamers), 121.0, 120.3, 119.4, 119.1 (C5' major, minor and rotamers), 65.6, 65.5, 65.4, 65.1 (C7 major, minor and rotamers), 64.7, 64.6, 64.3, 64.2 (C5 major, minor and rotamers), 64.0, 63.9, 63.3 (C2 major, minor and rotamers), 62.3, 62.2, 62.0 (OCH₂CH₃ major, minor and rotamers), 43.2, 42.9, 42.5, 42.2 (C2'' major, minor and rotamers), 34.5, 33.3, 32.0, 31.4 (C4), 14.3, 14.2 (OCH₂CH₃ major, minor and rotamers); m/z (ESI⁺) 560 and 562 ([M+Na]⁺, 100%); HRMS (ESI⁺) m/z calcd for C₂₁H₂₀O₇N₃BrNaS [M+Na]⁺ 560.0098 and 562.0078; found 560.0097 and 562.0072.

(5*R*)-5-(4-Bromobenzyloxycarbonyl)-2-(5-bromopyridin-2-yl)-1-(3-ethoxy-3-oxopropanoyl)-1,3-thiazolidine, 88c



Synthesised from **81c** (1 eqv. 1 mmol); yield (0.5 g, 80%); yellow oil; an inseparable mixture of 6.5 : 1 *cis* and *trans* diastereomers; $R_f = 0.23$ (petrol : EtOAc; 3 : 1); $\nu_{\max}/\text{cm}^{-1}$ 1736 (s, C=O), 1662 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): major *cis* isomer as a 1 : 0.3 mixture of rotamers; minor *trans* isomer as a 1 : 1 mixture of rotamers: δ 8.58 (d, $J = 2.1$ Hz, 1H, H6'), 7.93 (d, $J = 8.4$ Hz, 1H, H3'), 7.77 (dd, $J = 8.4, 2.2$ Hz, 1H, H4'), 7.50 (d, $J = 8.2$ Hz, 2H, H3a'), 7.24 (d, $J = 8.3$ Hz, 2H, H2a'), 6.38 (s, H2 *cis* minor rotamer), 6.15 (s, obscured, H2 *trans* minor rotamer), 6.15 (s, 1H, H2 *cis* major rotamer), 6.13 (s, H2 *trans* major rotamer), 5.18 (app q, $J = 12.3$ Hz, 2H, H7), 5.10 – 5.03 (m, 1H, H5), 4.26 – 4.04 (m, 2H, OCH_2CH_3), 3.37 (d, $J = 15.6$ Hz, 1H, H2''_B), 3.34 – 3.29 (m, 1H, H4_B), 3.24 – 3.20 (m, 1H, H4_A), 3.17 (d, $J = 15.6$ Hz, 1H, H2''_A), 1.23 (t, $J = 7.2$ Hz, 3H, OCH_2CH_3); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): major *cis* isomer, major rotamer δ 169.8 (C6), 166.4 (C3''), 165.6 (C1''), 157.9 (C2'), 150.8 (C6'), 140.1 (C4'), 134.2 (C1a'), 132.0 (C3a'), 130.0 (C2a'), 122.8, 122.3 (C3' and C4a'), 120.5 (C5'), 67.2 (C7), 66.9 (C5), 65.0 (C2), 61.9 (OCH_2CH_3), 42.1 (C2''), 31.9 (C4), 14.1 (OCH_2CH_3); m/z (ESI⁺) 571 (50%), 573 ([M+H]⁺, 100%) and 575 (50%); HRMS (ESI⁺) m/z calcd for $\text{C}_{21}\text{H}_{21}\text{O}_5\text{N}_2\text{Br}_2\text{S}$ [M+H]⁺ 570.9532, 572.9512 and 574.9493; found 570.9532, 572.9510 and 574.9486.

(5*R*)-5-(4-Bromobenzyloxycarbonyl)-1-(3-ethoxy-3-oxopropanoyl)-2-phenyl-1,3-thiazolidine, 88e



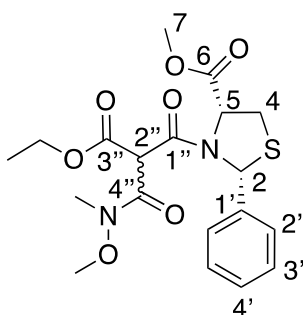
Synthesised from **81e** (1 eqv. 3.7 mmol); yield (1.1 g, 61%); yellow oil; an inseparable mixture of 2.2 : 1 *cis* and *trans* diastereomers; $R_f = 0.21$ (petrol : EtOAc; 4 : 1); $\nu_{\max}/\text{cm}^{-1}$ 1735 (s, C=O), 1660 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): major *cis* isomer as a 1 : 0.1 mixture of rotamers; minor *trans* isomer as a 1 : 0.2 mixture of rotamers: δ 7.56 – 7.63 (m, 4H, H2' and H3a' *trans*),

7.44 – 7.54 (m, 4H, H2' and H3a' *cis*), 7.27 – 7.40 (m, 6H, H4' and H3'), 7.17 – 7.25 (m, 4H, H2a'), 6.36 (s, H2 *cis* minor rotamer), 6.32 (s, H2 *trans* minor rotamer), 6.18 (s, 1H, H2 *trans* major rotamer), 6.14 (s, 1H, H2 *cis* major rotamer), 5.34 (app d, $J = 7.8$ Hz, 1H, H5 *trans*), 5.15 – 5.28 (m, 4H, H7), 5.10 (app t, $J = 6.8$ Hz, 1H, H5 *cis*), 4.23 (q, $J = 7.2$ Hz, 2H, OCH_2CH_3 *cis*), 4.05 (q, $J = 7.1$ Hz, 2H, OCH_2CH_3 *trans*), 3.07 – 3.54 (m, 8H, H4_A, H4_B, H2''_A, H2''_B), 1.29 (t, $J = 7.2$ Hz, 3H, OCH_2CH_3 *cis*), 1.19 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3 *trans*); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ 169.9, 169.6, 168.9 (C6 major, minor and rotamers), 166.8, 166.3 (C3'' major and minor), 165.7, 165.7 (C1'' major and minor), 141.8, 139.8 (C1' major and minor), 134.6, 134.4 (C1a' major and minor), 132.0, 131.9, 131.9, 131.7 (C3a' major, minor and rotamers), 130.2, 130.1, 129.9 (C2a' major, minor and rotamers), 129.4, 129.1 (C3' major and minor), 128.8, 128.7 (C4' major and minor), 127.2, 126.6, 125.1, 124.9 (C2' major, minor and rotamers), 122.7, 122.5 (C4a' major and minor), 67.4, 66.8, 66.5, 66.4 (C7 major and minor), 65.2, 65.0 (C5 major and minor), 64.7, 64.3 (C2 major and minor), 62.3, 61.8, 61.6 (OCH_2CH_3 major, minor and rotamers), 44.8, 42.5, 42.3, 41.6 (C2'' major and minor), 32.2, 31.4, 31.2, 29.4 (C4 major, minor and rotamers), 14.2, 14.2, 14.1 (OCH_2CH_3 major, minor and rotamers); m/z (ESI⁺) 492 and 494 ($[\text{M}+\text{H}]^+$, 100%); HRMS

(ESI⁺) *m/z* calcd for C₂₂H₂₃O₅NBrS [M+H]⁺ 492.0475 and 494.0455; found 492.0479 and 494.0457.

Tricarbonyl *N*-acylthiazolidines **92-99** were synthesised from the respective *N*-acylthiazolidines **24e** and **82-88** by general procedure **N** (section 6.4.3).

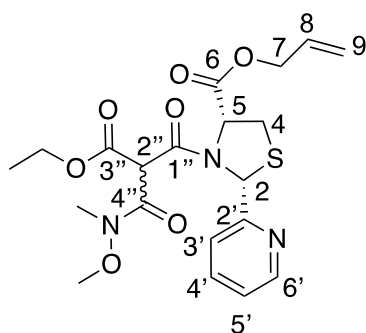
(2*R*,5*R*)-1-(3-Ethoxy-2-(methoxy(methyl)carbamoyl)-3-oxopropanoyl)-5-methoxycarbonyl-2-phenyl-1,3-thiazolidine, 92e



Synthesised from **24e** (1 eqv. 3.4 mmol); yield (1.1 g, 77%); pale yellow oil; an inseparable 1 : 1 C2'' *R/S* diastereomers; *R_f* = 0.20 (petrol : EtOAc; 3 : 2); $\nu_{\text{max}}/\text{cm}^{-1}$ 1745 (s, C=O), 1669 (s, C=O); ¹H NMR (CDCl₃, 400 MHz): δ 7.60 – 7.69 (m, 4H, H2'), 7.27 – 7.38 (m, 6H, H4' and H3'), 5.95 (s, 1H, H2, C2'' *R*), 5.77 (s, 1H, H2, C2''

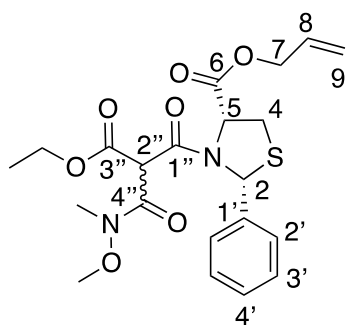
S), 5.15 (app q, *J* = 6.1 Hz, 2H, H5), 4.44 (s, 1H, H2'', C2'' *S*), 4.35 (s, 1H, H2'', C2'' *R*), 4.16 – 4.30 (m, 2H, OCH₂CH₃), 3.97 – 4.09 (m, 2H, OCH₂CH₃), 3.78 (s, 6H, H7), 3.31 (s, 3H, NOCH₃), 3.27 (s, 3H, NOCH₃), 3.09 – 3.31 (m, 4H, H4), 3.07 (s, 3H, NCH₃), 3.02 (s, 3H, NCH₃), 1.26 (t, *J* = 7.1 Hz, 3H, OCH₂CH₃), 1.13 (t, *J* = 7.1 Hz, 3H, OCH₂CH₃); ¹³C NMR (CDCl₃, 101 MHz): δ 170.1, 169.9 (C6), 165.2, 165.2 (C3''), 164.6, 164.0 (C1''), 163.9, 163.6 (C4''), 139.4, 139.2 (C1'), 129.0, 128.9 (C3'), 128.7 (C4'), 126.9, 126.5 (C2'), 66.1, 66.0 (C5), 65.1, 64.9 (C2), 62.3, 61.8 (OCH₂CH₃), 61.0, 60.9 (NOCH₃), 57.5, 56.8 (C2''), 52.7, 52.6 (C7), 32.6 (C4), 32.1, 32.0 (NCH₃), 14.1, 13.8 (OCH₂CH₃); *m/z* (ESI⁺) 425 ([M+H]⁺, 100%); HRMS (ESI⁺) *m/z* calcd for C₁₉H₂₅O₇N₂S [M+H]⁺ 425.1377; found 425.1378.

(2*R*,5*R*)-5-Allyloxycarbonyl-1-(3-ethoxy-2-(methoxy(methyl)carbamoyl)-3-oxopropanoyl)-2-(pyridin-2-yl)-1,3-thiazolidine, 93a



Synthesised from **82a** (1 eqv. 1.7 mmol); yield (364 mg, 47%); orange oil; an inseparable 0.8 : 1 C2'' *R/S* diastereomers; $R_f = 0.22$ (petrol : EtOAc; 2 : 3); $\nu_{\max}/\text{cm}^{-1}$ 1745 (s, C=O), 1670 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ 8.54 – 8.51 (m, 1H, H6 minor), 8.50 – 8.47 (m, 1H, H6' major), 8.10 (app t, $J = 8.1$ Hz, 2H, H3'), 7.78 – 7.68 (m, 2H, H4'), 7.22 (dd, $J = 8.4, 4.9$ Hz, 2H, H5'), 6.00 (s, 1H, H2 major, C2'' *S*), 5.95 (s, 1H, H2 minor, C2'' *R*), 5.94 – 5.82 (m, 2H, H8), 5.36 – 5.27 (m, 2H, H9_B), 5.24 – 5.19 (m, 2H, H9_A), 5.18 – 5.08 (m, 2H, H5), 4.66 (app t, $J = 5.8$ Hz, 4H, H7), 4.62 (s, 1H, H2'' minor, C2'' *R*), 4.48 (s, 1H, H2'' major, C2'' *S*), 4.30 – 4.16 (m, 2H, OCH_2CH_3 major), 4.02 (q, $J = 7.1$ Hz, 2H, OCH_2CH_3 minor), 3.48 (s, 3H, NOCH_3 major), 3.31 (s, 3H, NOCH_3 minor), 3.30 – 3.22 (m, 4H, H4), 3.13 (s, 3H, NCH_3 major), 3.01 (s, 3H, NCH_3 minor), 1.26 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3 major), 1.11 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3 minor); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ 169.5, 169.4 (C6), 164.9, 164.8 (C3''), 164.5, 164.0 (C1''), 163.8, 163.7 (C4''), 159.3, 159.0 (C2'), 149.5, 149.3 (C6'), 137.7, 137.3 (C4'), 131.4 (C8), 123.4 (C3'), 121.1 (C5'), 118.8 (C9), 67.4 (C7), 66.3 (C5), 65.1 (C2), 62.5, 61.9 (OCH_2CH_3), 61.2, 61.1 (NOCH_3), 57.2, 56.8 (C2''), 32.7, 32.2 (C4), 31.9, 31.8 (NCH_3), 14.0, 13.9 (OCH_2CH_3); m/z (ESI⁺) 452 ($[\text{M}+\text{H}]^+$, 100%); HRMS (ESI⁺) m/z calcd for $\text{C}_{20}\text{H}_{26}\text{O}_7\text{N}_3\text{S}$ $[\text{M}+\text{H}]^+$ 452.1486; found 452.1485.

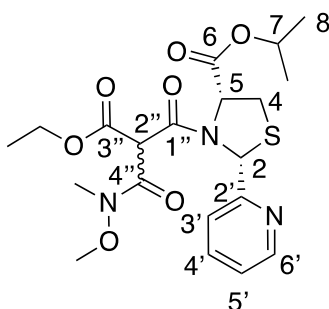
(2*R*,5*R*)-5-Allyloxycarbonyl-1-(3-ethoxy-2-(methoxy(methyl)carbamoyl)-3-oxopropanoyl)-2-phenyl-1,3-thiazolidine, 93e



Synthesised from **82e** (1 eqv. 3.3 mmol); yield (804 mg, 54%); pale yellow oil; an inseparable 1 : 1 C2'' *R/S* diastereomers; $R_f = 0.21$ (petrol : EtOAc; 3 : 2); $\nu_{\max}/\text{cm}^{-1}$ 1745 (s, C=O), 1670 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ 7.62 – 7.74 (m, 4H, H2'), 7.35 – 7.42 (m, 4H, H3'), 7.29 – 7.35 (m, 2H, H4'), 6.00 (s, 1H,

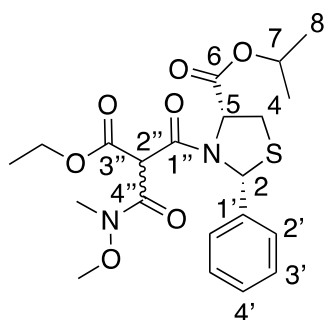
H2, C2'' *R*), 5.89 – 6.00 (m, 2H, H8), 5.82 (s, 1H, H2, C2'' *S*), 5.31 – 5.39 (m, 1H, H5), 5.38 (d, $J = 17.2$ Hz, 2H, H9_B), 5.28 (app dd, $J = 10.5, 1.2$ Hz, 2H, H9_A), 5.24 (dd, $J = 6.1, 3.5$ Hz, 1H, H5), 4.71 – 4.76 (m, 4H, H7), 4.49 (s, 1H, H2'', C2'' *S*), 4.39 (s, 1H, H2'', C2'' *R*), 4.23 – 4.37 (m, 2H, OCH_2CH_3), 4.09 (q, $J = 7.1$ Hz, 2H, OCH_2CH_3), 3.34 – 3.38 (m, 5H, NOCH_3 and H4_B), 3.31 (s, 3H, NOCH_3), 3.18 (dd, $J = 15.2, 3.5$ Hz, 2H, H4_A), 3.12 (s, 3H, NCH_3), 3.08 (s, 3H, NCH_3), 1.33 (t, $J = 7.2$ Hz, 3H, OCH_2CH_3), 1.18 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ 169.5, 169.4 (C6), 165.5 (C3''), 164.3 (C1''), 164.1, 163.8 (C4''), 139.6, 139.3 (C1'), 131.7 (C8), 129.2, 129.1 (C3'), 128.9 (C4'), 127.2, 126.7 (C2'), 119.0, 119.0 (C9), 66.5, 66.5, 66.4, 66.2 (C5 and C7), 65.4, 65.2 (C2), 62.6, 62.1 (OCH_2CH_3), 61.2, 61.1 (NOCH_3), 57.8, 57.1 (C2''), 32.9 (C4), 32.4, 32.3 (NCH_3), 14.3, 14.1 (OCH_2CH_3); m/z (ESI^+) 473 ($[\text{M}+\text{Na}]^+$, 100%); HRMS (ESI^+) m/z calcd for $\text{C}_{21}\text{H}_{26}\text{O}_7\text{N}_2\text{NaS}$ $[\text{M}+\text{Na}]^+$ 473.1353; found 473.1344.

(2*R*,5*R*)-1-(3-Ethoxy-2-(methoxy(methyl)carbamoyl)-3-oxopropanoyl)-5-isopropoxycarbonyl-2-(pyridin-2-yl)-1,3-thiazolidine, 94a



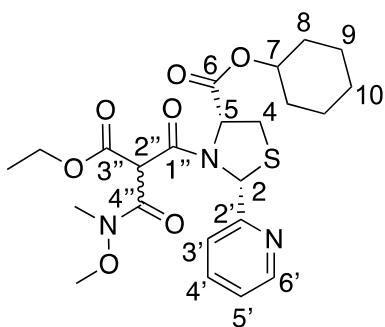
Synthesised from **83a** (1 eqv. 3.0 mmol); yield (740 mg, 54%); orange oil; an inseparable 0.7 : 1 C2'' *R/S* diastereomers; $R_f = 0.17$ (petrol : EtOAc; 2 : 3); $\nu_{\max}/\text{cm}^{-1}$ 1739 (s, C=O), 1668 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ 8.57 – 8.45 (m, 2H, H6'), 8.19 (d, $J = 7.7$ Hz, 2H, H3'), 7.81 – 7.64 (m, 2H, H4'), 7.29 – 7.20 (m, 2H, H5'), 6.02 (s, 1H, H2 major, C2'' *S*), 5.96 (s, 1H, H2 minor, C2'' *R*), 5.15 – 4.97 (m, 4H, H5 and H7), 4.62 (s, 1H, H2'' minor, C2'' *R*), 4.49 (s, 1H, H2'' major, C2'' *S*), 4.33 – 4.17 (m, 2H, OCH_2CH_3 major), 4.07 – 3.98 (m, 2H, OCH_2CH_3 minor), 3.67 (s, 3H, NOCH_3 minor), 3.50 (s, 3H, NOCH_3 major), 3.36 – 3.17 (m, 4H, H4), 3.14 (s, 3H, NCH_3 major), 3.03 (s, 3H, NCH_3 minor), 1.32 – 1.11 (m, 18H, OCH_2CH_3 and H8); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ 168.9, 168.7 (C6), 164.6, 164.6 (C3''), 163.5, 163.5 (C1''), 163.2 (C4''), 159.1, 158.9 (C2'), 149.1, 149.0 (C6'), 137.3, 137.0 (C4'), 123.1 (C3'), 120.7 (C5'), 69.2 (C7), 67.1, 67.1 (C5), 65.2, 65.2 (C2), 62.1, 61.4 (OCH_2CH_3), 60.9, 60.8 (NOCH_3), 56.8, 56.4 (C2''), 32.3, 31.8 (C4), 31.5, 31.4 (NCH_3), 21.3, 21.3 (C8), 13.7, 13.5 (OCH_2CH_3); m/z (ESI⁺) 454 ([$\text{M}+\text{H}$]⁺, 100%); HRMS (ESI⁺) m/z calcd for $\text{C}_{20}\text{H}_{28}\text{O}_7\text{N}_3\text{S}$ [$\text{M}+\text{H}$]⁺ 454.1642; found 454.1638.

(2*R*,5*R*)-1-(3-Ethoxy-2-(methoxy(methyl)carbamoyl)-3-oxopropanoyl)-5-isopropoxycarbonyl 2-phenyl-1,3-thiazolidine, 94e



Synthesised from **83e** (1 eqv. 4.0 mmol); yield (1.1 g, 61%); pale greenish yellow oil; an inseparable 0.8 : 1 C2'' *R/S* diastereomers; $R_f = 0.30$ (petrol : EtOAc; 1 : 1); $\nu_{\max}/\text{cm}^{-1}$ 1739 (s, C=O), 1668 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ 7.67 – 7.74 (m, 4H, H2'), 7.35 – 7.42 (m, 4H, H3'), 7.27 – 7.35 (m, 2H, H4'), 6.00 (s, 1H, H2, C2'' *R*), 5.81 (s, 1H, H2, C2'' *S*), 5.13 (app q, $J = 6.4$ Hz, 4H, H5 and H7), 4.49 (s, 1H, H2'', C2'' *S*), 4.41 (s, 1H, H2'', C2'' *R*), 4.23 – 4.36 (m, 2H, OCH_2CH_3), 4.08 (q, $J = 7.1$ Hz, 2H, OCH_2CH_3), 3.36 (s, 3H, NOCH_3), 3.32 (s, 3H, NOCH_3), 3.13 – 3.28 (m, 4H, H4), 3.12 (s, 3H, NCH_3), 3.08 (s, 3H, NCH_3), 1.23 – 1.37 (m, 15H, OCH_2CH_3 and H8), 1.17 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ 169.3, 169.1 (C6), 165.4 (C3''), 164.2 (C1''), 163.7 (C4''), 139.9, 139.6 (C1'), 129.1, 129.1 (C3'), 128.8 (C4'), 127.2, 126.7 (C2'), 69.8, 69.7 (C7), 66.4, 66.2 (C5), 65.8, 65.6 (C2), 62.6, 62.0 (OCH_2CH_3), 61.2, 61.1 (NOCH_3), 57.7, 57.1 (C2''), 32.4, 32.2 (NCH_3 and C4), 21.9 (C8), 14.3, 14.1 (OCH_2CH_3); m/z (ESI⁺) 453 ([$\text{M}+\text{H}$]⁺, 100%); HRMS (ESI⁺) m/z calcd for $\text{C}_{21}\text{H}_{29}\text{O}_7\text{N}_2\text{S}$ [$\text{M}+\text{H}$]⁺ 453.1690; found 453.1689.

(2*R*,5*R*)-5-Cyclohexyloxycarbonyl-1-(3-ethoxy-2-(methoxy(methyl)carbamoyl)-3-oxopropanoyl)-2-phenyl-1,3-thiazolidine, 95a



Yield (345 mg, 68%), from *cis*-**84a** (1 eqv. 1.0 mmol);

yellow oil; an inseparable 0.7 : 1 C2'' *R/S* diastereomers;

R_f = 0.28 (petrol : EtOAc; 2 : 3); $\nu_{\max}/\text{cm}^{-1}$ 1740 (s, C=O),

1669 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ 8.53 (d, J = 5.6

Hz, 1H, H6 minor), 8.50 (d, J = 5.6 Hz, 1H, H6' major), 8.17

(app dd, J = 7.9, 3.9 Hz, 2H, H3'), 7.78 – 7.68 (m, 2H, H4'), 7.22 (dd, J = 7.9, 5.6 Hz, 2H, H5'),

6.00 (s, 1H, H2 major, C2'' *S*), 5.94 (s, 1H, H2 minor, C2'' *R*), 5.11 – 5.00 (m, 2H, H5), 4.88 –

4.77 (m, 2H, H7), 4.60 (s, 1H, H2'' minor, C2'' *R*), 4.48 (s, 1H, H2'' major, C2'' *S*), 4.31 –

4.17 (m, 2H, OCH_2CH_3 major), 4.02 (q, J = 7.1 Hz, 2H, OCH_2CH_3 minor), 3.48 (s, 3H, NOCH_3

major), 3.32 (s, 3H, NOCH_3 minor), 3.30 – 3.15 (m, 4H, H4), 3.13 (s, 3H, NCH_3 major), 3.02

(s, 3H, 3H, NCH_3 minor), 1.92 – 1.77 (m, 4H, H8), 1.69 (br. s, 4H, H8), 1.55 – 1.30 (m, 12H,

H9 and H10), 1.26 (t, J = 7.1 Hz, 3H, OCH_2CH_3), 1.11 (t, J = 7.1 Hz, 3H, OCH_2CH_3); $^{13}\text{C NMR}$

(CDCl_3 , 101 MHz): δ 169.2, 169.0 (C6), 165.0, 164.9 (C3''), 163.8 (C1''), 163.5 (C4''), 159.5,

159.2 (C2'), 149.4, 149.3 (C6'), 137.6, 137.2 (C4'), 123.3 (C3'), 121.2, 121.1 (C5'), 74.4 (C7),

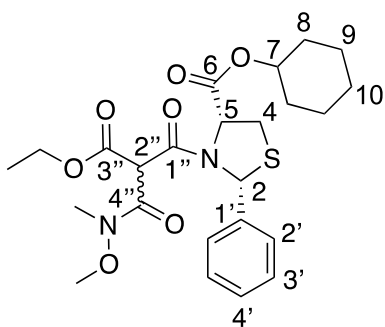
67.5, 67.5 (C5), 65.5, 65.5 (C2), 62.5, 61.8 (OCH_2CH_3), 61.2, 61.1 (NOCH_3), 57.2, 56.8 (C2),

32.7, 32.2 (C4), 31.9, 31.9 (NCH_3), 31.3 (C8), 25.3 (C10), 23.5 (C9), 14.0, 13.9 (OCH_2CH_3);

m/z (ESI⁺) 494 ([$\text{M}+\text{H}$]⁺, 100%); HRMS (ESI⁺) m/z calcd for $\text{C}_{23}\text{H}_{32}\text{O}_7\text{N}_3\text{S}$ [$\text{M}+\text{H}$]⁺ 494.1955;

found 494.1955.

(2*R*,5*R*)-5-Cyclohexyloxycarbonyl-1-(3-ethoxy-2-(methoxy(methyl)carbamoyl)-3-oxopropanoyl)-2-phenyl-1,3-thiazolidine, 95e



Synthesised from **84e** (1 eqv. 4.0 mmol); yield (1.0 g, 51%);

pale yellow oil; an inseparable 0.8 : 1 C2'' *R/S*

diastereomers, a mixture of rotamers; $R_f = 0.46$ (petrol :

EtOAc; 1 : 1); $\nu_{\max}/\text{cm}^{-1}$ 1741 (s, C=O), 1668 (s, C=O); ^1H

NMR (CDCl_3 , 400 MHz): δ 7.64 – 7.75 (m, 4H, H2'), 7.35 –

7.43 (m, 4H, H3'), 7.29 – 7.35 (m, 2H, H4'), 6.01 (s, 1H, H2, C2'' *R*), 5.82 (s, 1H, H2, C2'' *S*),

5.22 – 5.32 (m, 1H, H5), 5.16 (app q, $J = 6.1$ Hz, 1H, H5), 4.80 – 4.98 (m, 2H, H7), 4.49 (s,

1H, H2'', C2'' *S*), 4.40 (s, 1H, H2'', C2'' *R*), 4.25 – 4.33 (m, 2H, OCH_2CH_3), 4.09 (q, $J = 7.1$ Hz,

2H, OCH_2CH_3), 3.36 (s, 3H, NOCH_3), 3.32 (s, 3H, NOCH_3), 3.13 – 3.30 (m, 4H, H4), 3.12 (s,

3H, NCH_3), 3.08 (s, 3H, NCH_3), 1.81 – 2.00 (m, 4H, H8), 1.75 (br. s, 4H, H8), 1.43 – 1.61 (m,

6H, H9 and H10), 1.35 – 1.43 (m, 6H, H9 and H10), 1.33 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3), 1.18

(t, $J = 7.1$ Hz, 3H, OCH_2CH_3); ^{13}C NMR (CDCl_3 , 101 MHz): δ 169.6, 169.4, 169.2, 169.1 (C6),

165.5, 165.5, 165.0 (C3''), 164.2, 164.1 (C1''), 163.7 (C4''), 142.1, 139.9, 139.6 (C1'), 129.4,

129.4, 129.1, 129.1 (C3'), 128.8, 128.7 (C4'), 127.2, 126.7, 125.3, 125.1 (C2'), 75.6, 75.3,

74.6, 74.4 (C7), 66.4, 66.2 (C5), 65.8, 65.6 (C2), 62.6, 62.5, 62.1, 62.0 (OCH_2CH_3), 61.5,

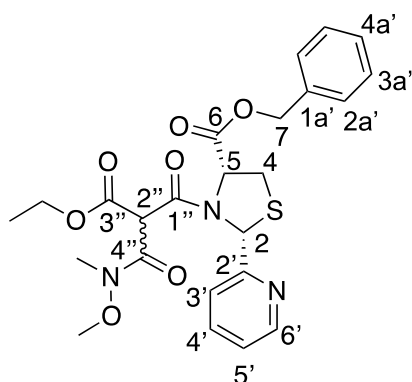
61.2, 61.1, 60.8 (NOCH_3), 57.8, 57.2, 57.1 (C2''), 34.4, 33.6, 33.2, 32.9 (C4), 32.5, 32.3

(NCH_3), 31.6, 31.6, 31.5, 31.5 (C8), 25.5, 25.5, 25.4, 25.3 (C10), 23.9, 23.8, 23.7, 23.6 (C9),

14.3, 14.3, 14.1, 14.0 (OCH_2CH_3); m/z (ESI⁺) 493 ([$\text{M}+\text{H}$]⁺, 100%); HRMS (ESI⁺) m/z calcd for

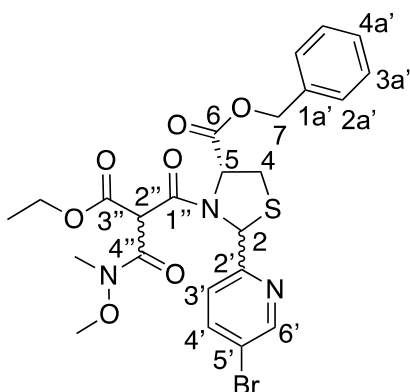
$\text{C}_{24}\text{H}_{33}\text{O}_7\text{N}_2\text{S}$ [$\text{M}+\text{H}$]⁺ 493.2003; found 493.2001.

(2*R*,5*R*)-5-Benzoyloxycarbonyl-1-(3-ethoxy-2-(methoxy(methyl)carbamoyl)-3-oxopropanoyl)-2-(pyridin-2-yl)-1,3-thiazolidine, 96a



Synthesised from **85a** (1 eqv. 1.4 mmol); yield (375 mg, 53%); bright yellow oil; an inseparable 0.7 : 1 C2'' *R/S* diastereomers; $R_f = 0.16$ (petrol : EtOAc; 2 : 3); $\nu_{\max}/\text{cm}^{-1}$ 1743 (s, C=O), 1663 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ 8.53 (d, $J = 4.6$ Hz, 1H, H6' minor), 8.49 (d, $J = 6.4$ Hz, 1H, H6' major), 8.03 (d, $J = 7.9$ Hz, 2H, H3'), 7.65 – 7.52 (m, 2H, H4'), 7.35 (app t, $J = 8.4$ Hz, 10H, H2a', H3a' and H4a'), 7.24 – 7.17 (m, 2H, H5'), 6.02 (s, 1H, H2 major, C2'' *S*), 5.98 (s, 1H, H2 minor, C2'' *R*), 5.26 – 5.14 (m, 6H, H5 and H7), 4.64 (s, 1H, H2'' minor, C2'' *R*), 4.51 (s, 1H, H2'' major, C2'' *S*), 4.36 – 4.15 (m, 2H, 2H, OCH_2CH_3 major), 4.05 (q, $J = 7.1$ Hz, 2H, OCH_2CH_3 minor), 3.50 (s, 3H, NOCH_3 major), 3.34 – 3.27 (m, 4H, H4), 3.26 (s, 3H, NOCH_3 minor), 3.16 (s, 3H, NCH_3 major), 3.04 (s, 3H, NCH_3 minor), 1.29 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3), 1.14 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ 169.7, 169.5 (C6), 165.0, 164.9 (C3''), 164.2 (C1''), 163.9 (C4''), 159.3, 159.1 (C2'), 149.5, 149.3 (C6'), 137.7, 137.3 (C4'), 135.2, 135.2 (C1a'), 128.7, 128.6, 128.5, 128.5, 128.4, 128.4 (C2a', C3a' and C4a'), 123.4 (C3'), 121.2 (C5'), 67.7, 67.6, 67.5 (C7 and C5), 65.3 (C2), 62.6, 62.0 (OCH_2CH_3), 61.3, 61.1 (NOCH_3), 57.3, 56.9 (C2''), 32.8, 32.2 (C4), 32.0, 31.9 (NCH_3), 14.1, 14.0 (OCH_2CH_3); m/z (ESI⁺) 502 ($[\text{M}+\text{H}]^+$, 100%); HRMS (ESI⁺) m/z calcd for $\text{C}_{24}\text{H}_{28}\text{O}_7\text{N}_3\text{S}$ $[\text{M}+\text{H}]^+$ 502.1642; found 502.1639.

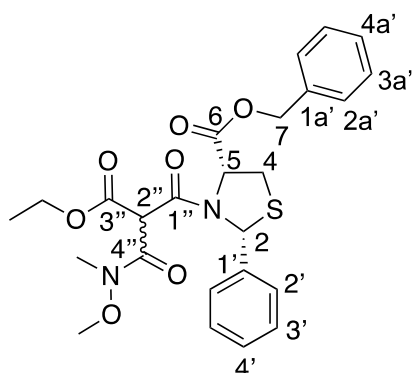
(5*R*)-5-Benzoyloxycarbonyl-2-(5-bromopyridin-2-yl)-1-(3-ethoxy-2-methoxy(methyl)carbamoyl)-3-oxopropanoyl)-1,3-thiazolidine, 96c



Synthesised from **85c** (1 eqv. 6.5 mmol); yield (1.5 g, 40%); yellow oil; an inseparable mixtures of diastereomers, 0.7 : 1 C2'' *R/S*, 2.1 : 1 *cis/trans*-2,5; R_f = 0.19 (petrol : EtOAc; 13 : 7); $\nu_{\max}/\text{cm}^{-1}$ 1742 (s, C=O), 1668 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ 8.61 – 8.51 (m, 2H, H6'), 7.96 (app t, J = 8.3 Hz, 2H, H3'), 7.79 – 7.64

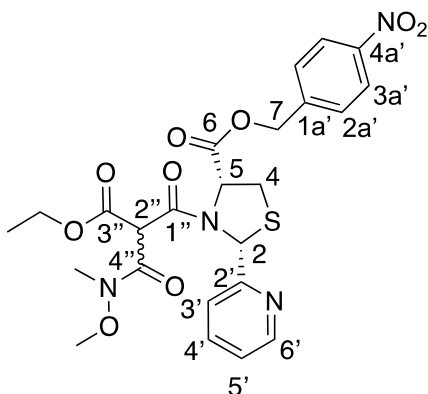
(m, 2H, H4'), 7.38 – 7.33 (m, 10H, H2a', H3a' and H4a'), 5.98 (s, 1H, H2 *cis*, C2'' *S*), 5.95 (s, 1H, H2 *cis*, C2'' *R*), 5.25 – 5.15 (m, 6H, H5 and H7), 4.64 (s, 1H, H2'', C2'' *R*), 4.52 (s, 1H, H2'', C2'' *S*), 4.33 – 4.17 (m, 2H, OCH_2CH_3), 4.16 – 4.03 (m, 2H, OCH_2CH_3), 3.58 (s, 3H, NOCH_3), 3.55 (s, 3H, NOCH_3), 3.42 – 3.21 (m, 4H, H4), 3.17 (s, 3H, NCH_3), 3.06 (s, 3H, NCH_3), 1.32 – 1.22 (m, 3H, OCH_2CH_3), 1.18 (t, J = 7.1 Hz, 3H, OCH_2CH_3); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ 169.8, 169.6, 169.6 (C6), 164.8, 164.4, 164.1, 163.9, 163.8, 163.7 (C3'', C1'' and C4''), 158.8, 157.9, 157.7 (C2'), 150.5, 150.5, 150.4 (C6'), 140.2, 139.8, 139.6, 139.4 (C4'), 135.2 (C1a'), 128.9, 128.8, 128.8, 128.7, 128.6, 128.5 (C2a', C3a' and C4a'), 122.8 (C3'), 120.9, 120.5 (C5'), 68.4, 67.8 (C6), 67.1, 67.0 (C5), 65.3, 63.9 (C2), 62.7, 62.2 (OCH_2CH_3), 61.4, 61.1 (NOCH_3), 58.3, 57.4, 57.0 (C2''), 33.5, 32.8 (C4), 32.1, 32.0 (NCH_3), 14.3, 14.2, 14.0 (OCH_2CH_3); m/z (ESI⁺) 602 and 604 ($[\text{M}+\text{Na}]^+$, 100%); HRMS (ESI⁺) m/z calcd for $\text{C}_{24}\text{H}_{26}\text{O}_7\text{N}_3\text{NaS}$ $[\text{M}+\text{Na}]^+$ 602.0567 and 604.0548; found 602.0565 and 604.0542.

(2*R*,5*R*)-5-Benzoyloxycarbonyl-1-(3-ethoxy-2-(methoxy(methyl)carbamoyl)-3-oxopropanoyl)-2-phenyl-1,3-thiazolidine, 96e



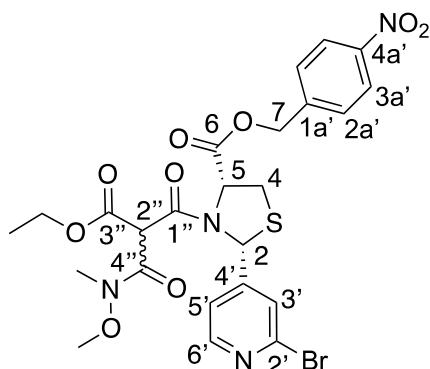
Synthesised from **85e** (1 eqv. 4.6 mmol); yield (1.3 g, 57%); colourless oil; an inseparable 0.8 : 1 C2'' *R/S* diastereomers; $R_f = 0.31$ (petrol : EtOAc; 1 : 1); $\nu_{\max}/\text{cm}^{-1}$ 1744 (s, C=O), 1671 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ 7.58 – 7.65 (m, 4H, H2'), 7.34 – 7.42 (m, 12H, H3', H3a', H2a'), 7.28 – 7.33 (m, 4H, H4' and H4a'), 5.99 (s, 1H, H2, C2'' *R*), 5.81 (s, 1H, H2, C2'' *S*), 5.20 – 5.32 (m, 6H, H5 and H7), 4.46 (s, 1H, H2'', C2'' *S*), 4.38 (s, 1H, H2'', C2'' *R*), 4.26 – 4.32 (q, $J = 7.2$ Hz, 2H, OCH_2CH_3), 4.09 (q, $J = 7.2$ Hz, 2H, OCH_2CH_3), 3.25 – 3.38 (obscured, 4H, H4), 3.34 (s, 3H, NOCH_3), 3.20 (s, 3H, NOCH_3), 3.07 (s, 3H, NCH_3), 3.12 (s, 3H, NCH_3), 1.32 (t, $J = 7.2$ Hz, 3H, OCH_2CH_3), 1.17 (t, $J = 7.2$ Hz, 3H, OCH_2CH_3); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ 169.7, 169.5 (C6), 165.4, 165.3 (C3''), 164.9, 164.3 (C1''), 164.1, 163.8 (C4''), 139.4, 139.1 (C1'), 135.3, 135.2 (C1a'), 129.1, 129.0 (C3'), 128.8, 128.7 (C4a' and C3a'), 128.6, 128.5 (C4' and C2a'), 127.1, 126.6 (C2'), 67.8, 67.7 (C7), 66.4, 66.2 (C5), 65.5, 65.3 (C2), 62.5, 62.0 (OCH_2CH_3), 61.0, 61.0 (NOCH_3), 57.7, 57.0 (C2''), 32.8, 32.4 (C4), 32.2, 32.2 (NCH_3), 14.2, 14.0 (OCH_2CH_3); m/z (ESI⁺) 523 ($[\text{M}+\text{Na}]^+$, 100%); HRMS (ESI⁺) m/z calcd for $\text{C}_{25}\text{H}_{28}\text{O}_7\text{N}_2\text{NaS}$ $[\text{M}+\text{Na}]^+$ 523.1509; found 523.1508.

(2*R*,5*R*)-1-(3-Ethoxy-2-(methoxy(methyl)carbamoyl)-5-(4-nitrobenzyloxycarbonyl)-3-oxopropanoyl)-2-(pyridin-2-yl)-1,3-thiazolidine, 97a



Synthesised from **86a** (1 eqv. 2.4 mmol); yield (745 mg, 57%); yellow oil; an inseparable 0.7 : 1 C2'' *R/S* diastereomers; $R_f = 0.23$ (petrol : EtOAc; 3 : 7); $\nu_{\max}/\text{cm}^{-1}$ 1747 (s, C=O), 1671 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ 8.54 (d, $J = 4.7$ Hz, 1H, H6' minor), 8.50 (d, $J = 4.7$ Hz, 1H, H6' major), 8.18 (d, $J = 8.7$ Hz, 4H, H3a'), 8.00 (d, $J = 7.9$ Hz, 2H, H3'), 7.67 (app dd, $J = 7.9, 1.7$ Hz, 2H, H4'), 7.56 (d, $J = 8.7$ Hz, 4H, H2a'), 7.23 (dd, $J = 7.9, 4.7$ Hz, 2H, H5'), 6.04 (s, 1H, H2 major, C2'' *S*), 6.02 (s, 1H, H2 minor, C2'' *R*), 5.39 – 5.25 (m, 4H, H7), 5.24 – 5.14 (m, 2H, H5), 4.69 (s, 1H, H2'' minor, C2'' *R*), 4.59 (s, 1H, H2'' major, C2'' *S*), 4.11 – 3.99 (m, 4H, OCH_2CH_3), 3.54 (s, 3H, NOCH_3 major), 3.43 – 3.31 (m, 4H, H4), 3.30 (s, 3H, NOCH_3 minor), 3.17 (s, 3H, NCH_3 major), 3.02 (s, 3H, NCH_3 minor), 1.28 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3 minor), 1.12 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3 major); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ 169.4, 169.3 (C6), 164.8, 164.6 (C3''), 164.2, 164.1 (C1''), 163.8, 163.7 (C4''), 159.0, 158.8 (C2'), 149.4, 149.3 (C6'), 147.5 (C4a'), 142.5 (C1a'), 137.5, 137.2 (C4'), 128.3, 128.2 (C3a'), 123.6 (C2a'), 123.4, 123.3 (C3'), 120.8, 120.7 (C5'), 67.3, 67.2 (C7), 65.8 (C5), 65.0, 64.9 (C2), 62.5, 61.8, (OCH_2CH_3) 61.2, 60.9 (NOCH_3), 57.1, 56.6 (C2''), 32.6, 32.1 (C4), 31.6, 31.6 (NCH_3), 13.9, 13.8 (OCH_2CH_3); m/z (ESI^+) 547 ($[\text{M}+\text{H}]^+$, 100%); HRMS (ESI^+) m/z calcd for $\text{C}_{24}\text{H}_{27}\text{O}_9\text{N}_4\text{S}$ $[\text{M}+\text{H}]^+$ 547.1493; found 547.1487.

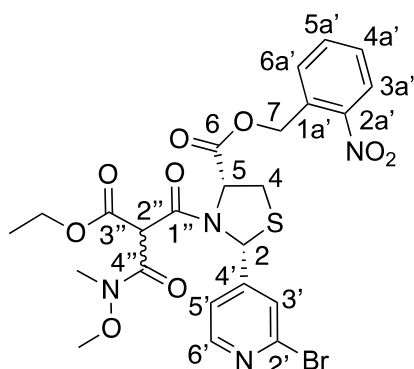
(2*R*,5*R*)-2-(2-Bromopyridin-4-yl)-1-(3-ethoxy-2-(methoxy(methyl)carbamoyl)-5-(4-nitrobenzyloxycarbonyl)-3-oxopropanoyl)-1,3-thiazolidine, 97b



Synthesised from **86b** (1 eqv. 4.0 mmol); yield (1.6 g, 65%); pale yellow oil; an inseparable 1 : 1 C2'' *R/S* diastereomers, a mixture of rotamers; $R_f = 0.26$ (petrol : EtOAc; 2 : 3); $\nu_{\max}/\text{cm}^{-1}$ 1747 (s, C=O), 1672 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 500 MHz): δ 8.36 (d, $J = 5.1$ Hz,

1H, H6'), 8.34 (d, $J = 5.1$ Hz, 1H, H6'), 8.27 – 8.21 (m, 4H, H3a'), 7.94 (s, 1H, H3'), 7.85 (s, 1H, H3'), 7.77 (s, H3' minor rotamer), 7.74 (s, H3' minor rotamer), 7.58 (d, $J = 8.5$ Hz, 4H, H2a'), 7.55 – 7.41 (m, 2H, H5'), 6.27 (s, H2 minor rotamer), 6.26 (s, H2 minor rotamer), 6.09 (s, 1H, H2, C2'' *S*), 5.89 (s, 1H, H2, C2'' *R*), 5.45 – 5.26 (m, 6H, H5 and H7), 4.49 (s, 1H, H2'', C2'' *R*), 4.48 (s, 1H, H2'', C2'' *S*), 4.29 – 4.13 (m, 4H, OCH_2CH_3), 3.64 (s, NOCH_3 minor rotamer), 3.62 (s, NOCH_3 minor rotamer), 3.55 (s, 3H, NOCH_3), 3.47 (s, 3H, NOCH_3), 3.38 (dd, $J = 12.5, 6.2$ Hz, 2H, H4_B), 3.23 – 3.15 (m, obscured, 2H, H4_A), 3.20 (s, NCH_3 minor rotamer), 3.13 (s, NCH_3 minor rotamer), 3.11 (s, 3H, NCH_3), 3.01 (s, 3H, NCH_3), 1.30 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3), 1.23 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3); $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 126 MHz): δ 169.4, 169.2 (C6), 165.5, 164.7, 164.2, 163.8, 163.5 (C3'', C1'' and C4''), 152.3, 151.9 (C6'), 150.7, 150.7, 150.1 (C4'), 148.1, 148.1, 148.0 (C4a'), 143.0, 142.8, 142.3, 142.3, 141.9, 141.8 (C1a' and C2'), 128.9, 128.8, 128.7, 128.6 (C3a'), 126.2, 126.0, 125.5, 125.4 (C3'), 124.0 (C2a'), 121.2, 121.2, 120.6, 120.5 (C5'), 66.7, 66.5, 66.3, 66.2, 66.1, 65.9, 65.8, 65.5 (C7 and C5), 64.2, 64.2, 64.1 (C2), 63.0, 62.8, 62.7 (OCH_2CH_3), 61.4, 61.3 (NOCH_3), 59.9, 59.2, 58.1, 57.3 (C2''), 35.3, 32.5, 32.4, 32.3, 32.0, 31.7 (C4 and NCH_3), 14.2, 14.2, 14.1 (OCH_2CH_3); m/z (ESI⁺) 625 and 627 ([$\text{M}+\text{H}$]⁺, 100%); HRMS (ESI⁺) m/z calcd for $\text{C}_{24}\text{H}_{26}\text{O}_9\text{N}_4\text{BrS}$ [$\text{M}+\text{H}$]⁺ 625.0598 and 627.0580; found 625.0595 and 627.0572.

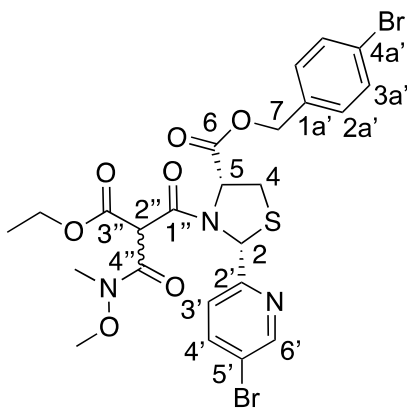
(2*R*,5*R*)-2-(2-Bromopyridin-4-yl)-1-(3-ethoxy-2-(methoxy(methyl)carbamoyl)-5-(2-nitrobenzyloxycarbonyl)-3-oxopropanoyl)-1,3-thiazolidine, 98b



Synthesised from **87b** (1 eqv. 2.4 mmol); yield (0.6 g, 40%); yellow oil; an inseparable 1 : 1 C2'' *R/S* diastereomers, a mixture of rotamers; $R_f = 0.25$ (petrol : EtOAc; 2 : 3); $\nu_{\max}/\text{cm}^{-1}$ 1746 (s, C=O), 16721(s, C=O); ^1H NMR (CDCl_3 , 500 MHz): δ 8.38 (d, $J = 5.2$ Hz, H6' minor rotamer), 8.35 (d, $J = 5.1$ Hz, H6' minor rotamer), 8.23 (d, $J = 5.1$ Hz, 2H, H6'), 8.13 (d, $J = 8.2$ Hz, 2H, H3a'), 7.94 (s, 1H, H3'), 7.84 (s, 1H, H3'), 7.77 – 7.47 (m, 6H, H4a', H5a' and H6a'), 7.42 – 7.36 (m, 2H, H5'), 6.27 (s, H2 minor rotamer), 6.25 (s, H2 minor rotamer), 6.11 (s, 1H, H2, C2'' *S*), 5.86 (s, 1H, H2, C2'' *R*), 5.69 (br. s, 4H, H7), 5.66 – 5.55 (m, 2H, H5), 4.51 (s, 1H, H2'', C2'' *R*), 4.48 (s, 1H, H2'', C2'' *S*), 4.31 – 4.14 (m, 4H, OCH_2CH_3), 3.65 (s, NOCH_3 minor rotamer), 3.64 (s, NOCH_3 minor rotamer), 3.55 (s, 3H, NOCH_3), 3.47 (s, 3H, NOCH_3), 3.43 – 3.36 (m, 2H, H4_B), 3.26 – 3.21 (m, 2H, H4_A), 3.20 (s, NCH_3 minor rotamer), 3.16 (s, NCH_3 minor rotamer), 3.11 (s, 3H, NCH_3), 2.99 (s, 3H, NCH_3), 1.34 – 1.20 (m, 6H, OCH_2CH_3); $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 126 MHz): δ 169.3, 169.2, 169.1 (C6), 165.3, 164.8, 164.6, 164.4, 164.2, 163.8, 163.5 (C3'', C1'' and C4''), 152.4, 151.9 (C6'), 150.8, 150.7, 150.0 (C4'), 147.5 (C2a'), 143.0, 142.8, 142.3 (C2'), 134.2, 134.2, 134.1, 134.0 (C6a'), 131.4, 131.4, 130.7, 130.6 (C1a'), 129.9, 129.7, 129.7, 129.6 (C5a'), 129.5, 129.5, 129.2, 129.2 (C4a'), 126.3, 125.6, 125.5, 125.2 (C3' and C3a'), 121.2, 121.2, 120.6, 120.5 (C5'), 66.0, 65.9, 65.8, 65.5, 65.2, 65.1, 64.7, 64.6, 64.2, 64.0 (C7, C5 and C2), 63.0, 63.0, 62.7, 62.7 (OCH_2CH_3), 61.6, 61.5, 61.4, 61.3 (NOCH_3), 59.6, 58.8, 58.2, 57.4 (C2''), 35.1, 33.5, 32.6, 32.4, 32.3, 32.0, 31.8 (C4 and NCH_3), 14.3, 14.2, 14.1 (OCH_2CH_3); m/z (ESI⁺) 625 and 627 ([M+H]⁺,

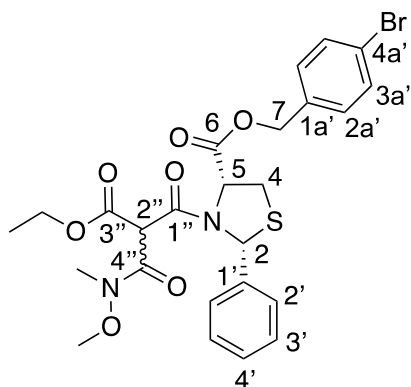
100%); HRMS (ESI⁺) *m/z* calcd for C₂₄H₂₆O₉N₄BrS [M+H]⁺ 625.0598 and 627.0580; found 625.0594 and 627.0572.

(2*R*,5*R*)-5-(4-Bromobenzyloxycarbonyl)-2-(5-bromopyridin-2-yl)-1-(3-ethoxy-2-(methoxy(methyl)carbamoyl)-3-oxopropanoyl)-1,3-thiazolidine, 99c



Synthesised from **88c** (1 eqv. 0.5 mmol); yield (200 mg, 69%); pale yellow oil; an inseparable 0.7 : 1 C2'' *R/S* diastereomers; *R_f* = 0.17 (petrol : EtOAc; 3 : 2); *v*_{max}/cm⁻¹ 1746 (s, C=O), 1672 (s, C=O); ¹H NMR (CDCl₃, 500 MHz): δ 8.57 (d, *J* = 2.1 Hz, 1H, H6'), 8.54 (d, *J* = 2.1 Hz, 1H, H6'), 7.95 (app dd, *J* = 12.1, 8.4 Hz, 2H, H3'), 7.76 (dd, *J* = 8.4, 2.3 Hz, 1H, H4'), 7.73 (dd, *J* = 8.4, 2.3 Hz, 1H, H4'), 7.49 (dd, *J* = 8.5, 2.1 Hz, 4H, H3a'), 7.24 (d, *J* = 8.5 Hz, 4H, H2a'), 5.97 (s, 1H, H2, C2'' *S*), 5.95 (s, 1H, H2, C2'' *R*), 5.23 – 5.08 (m, 6H, H7 and H5), 4.64 (s, 1H, H2'', C2'' *R*), 4.53 (s, 1H, H2'', C2'' *S*), 4.31 – 4.18 (m, 2H, OCH₂CH₃), 4.08 (q, *J* = 7.1 Hz, 2H, OCH₂CH₃), 3.60 (s, 3H, NOCH₃), 3.56 (s, 3H, NOCH₃), 3.37 – 3.19 (m, 4H, H4), 3.17 (s, 3H, NCH₃), 3.05 (s, 3H, NCH₃), 1.29 (t, *J* = 7.1 Hz, 3H, OCH₂CH₃), 1.17 (t, *J* = 7.1 Hz, 3H, OCH₂CH₃); ¹³C NMR (CDCl₃, 101 MHz): δ 169.7, 169.6 (C6), 164.9, 164.8 (C3''), 164.1, 163.9 (C1''), 163.8 (C4''), 157.9, 157.6 (C2'), 150.6, 150.4 (C6'), 140.2, 139.8 (C4'), 134.2 (C1a'), 131.9 (C3a'), 130.1 (C2a'), 122.7, 122.7 (C3' and C4a'), 120.6, 120.5 (C5'), 67.2, 67.0, 67.0, 66.9 (C7 and C5), 65.3, 65.2 (C2), 62.7, 62.2 (OCH₂CH₃), 61.4, 61.1 (NOCH₃), 57.4, 56.9 (C2''), 34.0 (C4), 32.0 (NCH₃), 14.2, 14.0 (OCH₂CH₃); *m/z* (ESI⁺) 658, 660 ([M+H]⁺, 100%) and 662; HRMS (ESI⁺) *m/z* calcd for C₂₄H₂₆O₇N₃Br₂S [M+H]⁺ 657.9853, 659.9833 and 661.9815; found 657.9847, 659.9826 and 661.9804.

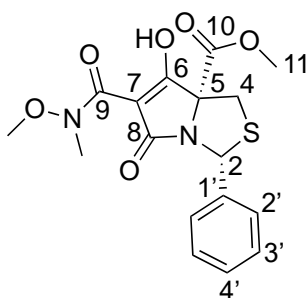
(2*R*,5*R*)-5-(4-Bromobenzoyloxycarbonyl)-1-(3-ethoxy-2-(methoxy(methyl)carbamoyl)-3-oxopropanoyl)-2-phenyl-1,3-thiazolidine, 99e



Synthesised from **88e** (1 eqv. 3.5 mmol); yield (1.0 g, 49%); yellow oil; an inseparable 1 : 1 C2'' *R/S* diastereomers; $R_f = 0.17$ (petrol : EtOAc; 3 : 2); $\nu_{\max}/\text{cm}^{-1}$ 1742 (s, C=O), 1668 (s, C=O); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ 7.62 (app dd, $J = 6.6, 2.9$ Hz, 2H, H2'), 7.59 (app dd, $J = 6.4, 3.0$ Hz, 2H, H2'), 7.50 (dd, $J = 8.4, 1.7$ Hz, 4H, H3a'), 7.31 – 7.35 (m, 4H, H3'), 7.28 (app dd, $J = 8.4, 3.1$ Hz, 6H, H2a' and H4'), 5.99 (s, 1H, H2, C2'' *R*), 5.82 (s, 1H, H2, C2'' *S*), 5.14 – 5.27 (m, 6H, H5 and H7), 4.46 (s, 1H, H2'', C2'' *S*), 4.37 (s, 1H, H2'', C2'' *R*), 4.24 – 4.33 (m, 2H, OCH_2CH_3), 4.09 (q, $J = 7.1$ Hz, 2H, OCH_2CH_3), 3.24 – 3.30 (m, 4H, H4), 3.34 (s, 3H, NOCH_3), 3.21 (s, 3H, NOCH_3), 3.14 (s, 3H, NCH_3), 3.08 (s, 3H, NCH_3), 1.32 (t, $J = 7.2$ Hz, 3H, OCH_2CH_3), 1.17 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ 169.7, 169.5 (C6), 165.5, 165.3 (C1''), 164.4, 164.1 (C3''), 163.9 (C4''), 139.4, 139.2 (C1'), 134.4, 134.3 (C1a'), 131.9 (C3a'), 130.2, 130.1 (C2a'), 129.2, 129.1 (C3'), 128.9 (C4'), 127.1, 126.6 (C2'), 122.7, 122.6 (C4a'), 66.9, 66.9 (C7), 66.4, 66.2 (C5), 65.5, 65.3 (C2), 62.6, 62.1 (OCH_2CH_3), 61.1, 61.1 (NOCH_3), 57.7, 57.0 (C2''), 32.9, 32.4 (C4), 32.2, 32.3 (NCH_3), 14.3, 14.1 (OCH_2CH_3); m/z (ESI⁺) 601 and 603 ($[\text{M}+\text{Na}]^+$, 100%); HRMS (ESI⁺) m/z calcd for $\text{C}_{25}\text{H}_{27}\text{O}_7\text{N}_2\text{BrNaS}$ $[\text{M}+\text{Na}]^+$ 601.0615, 603.0596; found 601.0611 and 603.0591.

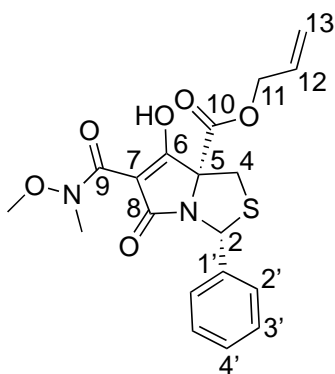
Tetramate Weinreb amides **101-109** were synthesised from the respective tricarbonyl *N*-acylthiazolidines **92-99** by general procedure **O** (section 6.4.4).

(2*R*,5*R*)-1-Aza-6-hydroxy-5-methoxycarbonyl-7-(methoxy(methyl)carbamoyl)-8-oxo-2-phenyl-3-thiabicyclo[3.3.0]-oct-6-ene, 101e



Synthesised from **92e** (1 eqv. 2.6 mmol); yield (912 mg, 93%); pale yellow foamy oil; $R_f = 0.31$ (EtOAc : MeOH; 9 : 1); ^1H NMR (CDCl_3 , 400 MHz): δ (*OH* not observed), 7.51 (d, $J = 6.8$ Hz, 2H, H_2'), 7.28 – 7.36 (m, 3H, H_3' and H_4'), 6.32 (s, 1H, H_2), 3.90 (d, $J = 11.5$ Hz, 1H, $\text{H}_{4\text{B}}$), 3.80 (s, 3H, NOCH_3), 3.69 (s, 3H, H_{11}), 3.51 (br. s, 3H, NCH_3), 3.12 (d, $J = 11.5$ Hz, 1H, $\text{H}_{4\text{A}}$); ^{13}C NMR (CDCl_3 , 101 MHz): δ 185.5 (C6), 176.9 (C8), 167.9 (C10), 166.3 (C9), 138.9 (C1'), 128.5 (C3'), 128.3 (C4'), 126.9 (C2'), 80.4 (C7), 64.7 (C5), 62.3 (C2), 61.2 (NOCH_3), 53.5 (C11), 35.7 (C4), 35.3 (NCH_3); m/z (ESI $^+$) 379 ([$\text{M}+\text{H}$] $^+$, 100%); HRMS (ESI $^+$) m/z calcd for $\text{C}_{17}\text{H}_{19}\text{O}_6\text{N}_2\text{S}$ [$\text{M}+\text{H}$] $^+$ 379.0958; found 379.0959.

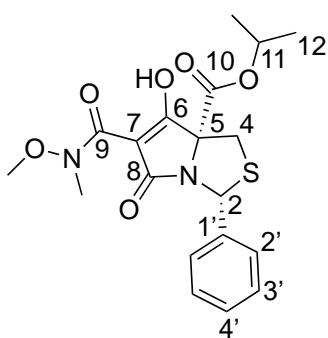
(2*R*,5*R*)-5-Allyloxycarbonyl-1-aza-6-hydroxy-7-(methoxy(methyl)carbamoyl)-8-oxo-2-phenyl-3-thiabicyclo[3.3.0]-oct-6-ene, 102e



Synthesised from **93e** (1 eqv. 1.7 mmol); yield (590 mg, 86%); yellow oil; $R_f = 0.21$ (EtOAc : MeOH; 9 : 1); ^1H NMR (CDCl_3 , 400 MHz): δ (*OH* not observed), 7.51 (d, $J = 7.0$ Hz, 2H, H_2'), 7.25 – 7.36 (m, 3H, H_3' and H_4'), 6.32 (s, 1H, H_2), 5.67 – 5.83 (m, 1H, H_{12}), 5.24 (dd, $J = 17.2, 1.4$ Hz, 1H, $\text{H}_{13\text{B}}$), 5.19 (dd, $J = 10.4, 1.4$ Hz, 1H, $\text{H}_{13\text{A}}$), 4.58 (d, $J = 5.8$ Hz, 2H, H_{11}), 3.91 (d, $J = 11.5$ Hz, 1H, $\text{H}_{4\text{B}}$), 3.80 (s, 3H, NOCH_3), 3.51 (br. s, 3H, NCH_3), 3.12 (d, $J = 11.5$ Hz, 1H, $\text{H}_{4\text{A}}$); ^{13}C NMR (CDCl_3 , 101 MHz): δ

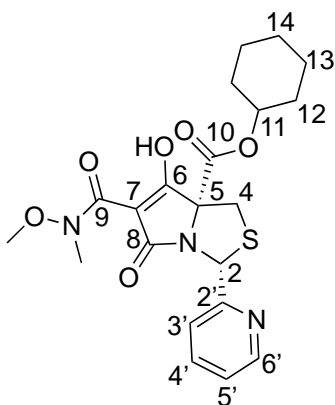
185.6 (C6), 177.0 (C8), 167.2 (C10), 166.3 (C9), 139.0 (C1'), 131.2 (C12), 128.5 (C3'), 128.2 (C4'), 127.0 (C2'), 119.2 (C13), 80.5 (C7), 67.2 (C11), 64.8 (C5), 62.3 (C2), 61.2 (NOCH₃), 35.7 (C4), 35.2 (NCH₃); *m/z* (ESI⁺) 405 ([M+H]⁺, 100%); HRMS (ESI⁺) *m/z* calcd for C₁₉H₂₁O₆N₂S [M+H]⁺ 405.1115; found 405.1115.

(2*R*,5*R*)-1-Aza-6-hydroxy-5-isopropoxycarbonyl-7-(methoxy(methyl)carbamoyl)-8-oxo-2-phenyl-3-thiabicyclo[3.3.0]-oct-6-ene, 103e



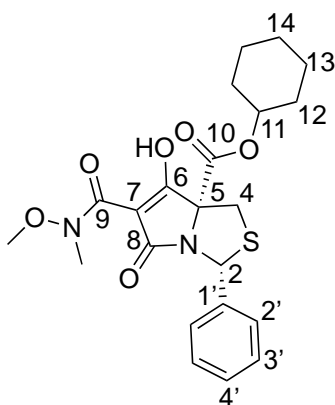
Synthesised from **94e** (1 eqv. 2.2 mmol); yield (760 mg, 85%); yellow oil; *R_f* = 0.23 (EtOAc : MeOH; 9 : 1); ¹H NMR (CDCl₃, 400 MHz): δ (*OH* not observed), 7.53 (d, *J* = 6.4 Hz, 2H, H2'), 7.25 – 7.35 (m, 3H, H3' and H4'), 6.32 (s, 1H, H2), 5.00 (sept, *J* = 6.3 Hz, 1H, H11), 3.90 (d, *J* = 11.5 Hz, 1H, H4_B), 3.80 (s, 3H, NOCH₃), 3.52 (br. s, 3H, NCH₃), 3.10 (d, *J* = 11.5 Hz, 1H, H4_A), 1.16 (d, *J* = 6.3 Hz, 3H, H12_B), 1.10 (d, *J* = 6.3 Hz, 3H, H12_A); ¹³C NMR (CDCl₃, 101 MHz): δ 185.9 (C6), (C8 not shown), 166.5 (C10), 166.4 (C9), 139.0 (C1'), 128.5 (C3'), 128.2 (C4'), 127.0 (C2'), 80.4 (C7), 70.9 (C11), 64.6 (C5), 62.4 (NOCH₃ and C2), 35.6 (NCH₃ and C4), 21.5 (C12_B), 21.4 (C12_A); *m/z* (ESI⁻) 405 ([M-H]⁻, 100%); HRMS (ESI⁻) *m/z* calcd for C₁₉H₂₁O₆N₂S [M-H]⁻ 405.1126; found 405.1123.

(2*R*,5*R*)-1-Aza-5-cyclohexyloxycarbonyl-6-hydroxy-7-(methoxy(methyl)carbamoyl)-8-oxo-2-(pyridin-2-yl)-3-thiabicyclo[3.3.0]-oct-6-ene, 104a



Synthesised from **95a** (1 eqv. 0.7 mmol); yield (100 mg, 32%); yellow foamy oil; $R_f = 0.12$ (EtOAc : MeOH; 9 : 1); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ (*OH* not observed), 8.52 (d, $J = 4.0$ Hz, 1H, H6'), 7.74 – 7.58 (m, 2H, H3' and H4'), 7.18 (app t, $J = 5.3$ Hz, 1H, H5'), 6.38 (s, 1H, H2), 4.86 – 4.74 (m, 1H, H11), 3.93 (d, $J = 11.5$ Hz, 1H, H4_B), 3.82 (s, 3H, NOCH_3), 3.53 (br. s, 3H, NCH_3), 3.15 (d, $J = 11.5$ Hz, 1H, H4_A), 1.80 – 1.69 (m, 2H, H12), 1.69 – 1.59 (m, 2H, H12), 1.52 – 1.43 (m, 2H, H13), 1.41 – 1.29 (m, 4H, H13 and H14); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ (C6, C8 not shown), 166.5 (C10 and C9), 158.8 (C2'), 149.2 (C6'), 137.0 (C4'), 122.9 (C3'), 120.3 (C5'), 81.1 (C7), 75.5 (C11), 65.2 (C5), 62.5 (NOCH_3 and C2), 35.7 (C4 and NCH_3), 31.2 (C12), 25.3 (C14), 23.6 (C13); m/z (ESI⁻) 446 ($[\text{M}-\text{H}]^-$, 100%); HRMS (ESI⁻) m/z calcd for $\text{C}_{21}\text{H}_{24}\text{N}_3\text{O}_6\text{S}$ $[\text{M}-\text{H}]^-$ 446.1391; found 446.1400.

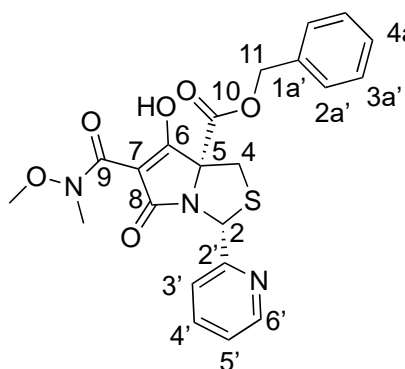
(2*R*,5*R*)-1-Aza-5-cyclohexyloxycarbonyl-6-hydroxy-7-(methoxy(methyl)carbamoyl)-8-oxo-2-phenyl-3-thiabicyclo[3.3.0]-oct-6-ene, 104e



Synthesised from **95e** (1 eqv. 2.0 mmol); yield (570 mg, 63%); yellow foamy oil; $R_f = 0.26$ (EtOAc : MeOH; 9 : 1); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ (*OH* not observed), 7.53 (d, $J = 6.9$ Hz, 2H, H2'), 7.27 – 7.34 (m, 3H, H3' and H4'), 6.32 (s, 1H, H2), 4.70 – 4.85 (m, 1H, H11), 3.91 (d, $J = 11.5$ Hz, 1H, H4_B), 3.81 (s, 3H, NOCH_3), 3.53 (br. s, 3H, NCH_3), 3.11 (d, $J = 11.5$ Hz, 1H, H4_A), 1.59 – 1.78 (m, 4H, H12), 1.20 – 1.39 (m, 6H, H13 and H14); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ

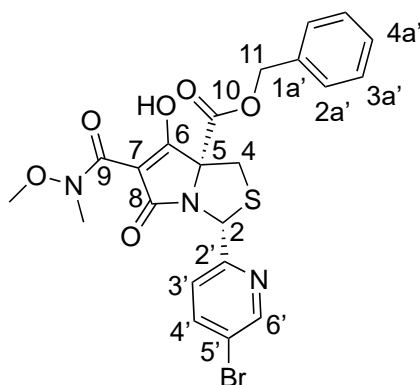
185.8 (C6), 176.6 (C8), 166.4 (C10), 166.2 (C9), 138.9 (C1'), 128.3 (C3'), 128.1 (C4'), 126.9 (C2'), 80.4 (C7), 75.3 (C11), 64.5 (C5), 62.2 (NOCH₃ and C2), 35.4 (C4 and NCH₃), 31.0 (C12), 31.0 (C12), 25.2 (C14), 23.4 (C13); *m/z* (ESI⁺) 447 ([M+H]⁺, 100%); HRMS (ESI⁺) *m/z* calcd for C₂₂H₂₇O₆N₂S [M+H]⁺ 447.1584; found 447.1583.

(2*R*,5*R*)-1-Aza-5-benzyloxycarbonyl-6-hydroxy-7-(methoxy(methyl)carbamoyl)-8-oxo-2-(pyridin-2-yl)-3-thiabicyclo[3.3.0]-oct-6-ene, 105a



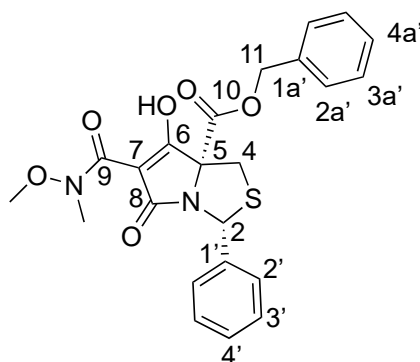
Synthesised from **96a** (1 eqv. 0.8 mmol); yield (95 mg, 28%); brown oil; *R_f* = 0.17 (EtOAc : MeOH; 9 : 1); ¹H NMR (CDCl₃, 400 MHz): δ (*OH* not observed), 8.40 (d, *J* = 4.7 Hz, 1H, H6'), 7.49 – 7.43 (m, 2H, H3' and H4'), 7.32 – 7.27 (m, 3H, H3a' and H4a'), 7.27 – 7.20 (m, 2H, H2a'), 7.14 – 7.08 (m, 1H, H5'), 6.39 (s, 1H, H2), 5.24 – 5.13 (m, 2H, H11), 3.99 (d, *J* = 11.6 Hz, 1H, H4_B), 3.80 (s, 3H, NOCH₃), 3.52 (br. s, 3H, NCH₃), 3.22 (d, *J* = 11.6 Hz, 1H, H4_A); ¹³C NMR (CDCl₃, 101 MHz): δ 186.4 (C6), 176.7 (C8), 167.1 (C10), 166.4 (C9), 158.6 (C2'), 149.1 (C6'), 137.1 (C4'), 135.2 (C1a'), 128.7 (C3a'), 128.5 (C4a'), 128.4 (C2a'), 122.9 (C3'), 120.2 (C5'), 81.3 (C7), 68.3 (C11), 65.4 (C5), 62.4 (NOCH₃ and C2), 35.8 (C4), 35.5 (NCH₃); *m/z* (ESI⁻) 454 ([M-H]⁻, 100%); HRMS (ESI⁻) *m/z* calcd for C₂₂H₂₀O₆N₃S [M-H]⁻ 454.1078; found 454.1066.

(2*R*,5*R*)-1-Aza-5-benzyloxycarbonyl-2-(5-bromopyridin-2-yl)-6-hydroxy-7-(methoxy(methyl)carbamoyl)-8-oxo-3-thiabicyclo[3.3.0]-oct-6-ene, 105c



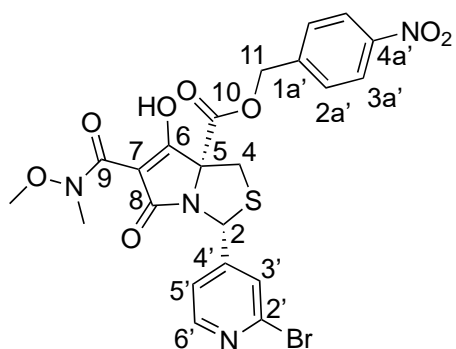
Synthesised from **96c** (1 eqv. 2.6 mmol); yield (550 mg, 40%); yellow oil; $R_f = 0.23$ (EtOAc : MeOH; 9 : 1); ^1H NMR (CDCl_3 , 400 MHz): δ (*OH* not observed), 8.33 (d, $J = 2.1$ Hz, 1H, H_{6'}), 7.48 (dd, $J = 8.4, 2.3$ Hz, 1H, H_{4'}), 7.36 – 7.25 (m, 4H, H_{3'}, H_{3a'} and H_{4a'}), 7.22 (d, $J = 6.6$ Hz, 2H, H_{2a'}), 6.29 (s, 1H, H₂), 5.24 (d, $J = 12.2$ Hz, 1H, H_{11B}), 5.11 (d, $J = 12.2$ Hz, 1H, H_{11A}), 3.95 (d, $J = 11.6$ Hz, 1H, H_{4B}), 3.79 (s, 3H, NOCH_3), 3.50 (br. s, 3H, NCH_3), 3.18 (d, $J = 11.6$ Hz, 1H, H_{4A}); ^{13}C NMR (CDCl_3 , 101 MHz): δ 186.2 (C₆), (C₈ not shown), 167.0 (C₁₀), 166.2 (C₉), 157.0 (C_{2'}), 150.1 (C_{6'}), 139.3 (C_{4'}), 134.9 (C_{1a'}), 128.6 (C_{2a'}, C_{4a'} and C_{3a'}), 122.5 (C_{3'}), 119.7 (C_{5'}), 81.0 (C₇), 68.4 (C₁₁), 64.7 (C₅), 63.5 (C₂), 62.5 (NOCH_3), 35.8 (C₄), 33.7 (NCH_3); m/z (ESI⁺) 532 and 534 ($[\text{M}-\text{H}]^+$, 100%); $\text{C}_{22}\text{H}_{19}\text{O}_6\text{N}_3\text{SBr}$ ($[\text{M}-\text{H}]^+$).

(2*R*,5*R*)-1-Aza-5-benzyloxycarbonyl-6-hydroxy-7-(methoxy(methyl)carbamoyl)-8-oxo-2-phenyl-3-thiabicyclo[3.3.0]-oct-6-ene, 105e



Synthesised from **96e** (1 eqv. 2.4 mmol); yield (764 mg, 70%); yellow oil; $R_f = 0.31$ (EtOAc : MeOH; 9 : 1); ^1H NMR (CDCl_3 , 400 MHz): δ (*OH* not observed), 7.41 – 7.56 (m, 2H, H_{2'}), 7.29 – 7.43 (m, 7H, H_{4a'}, H_{3'}, H_{3a'}, H_{2a'}), 7.19 – 7.34 (m, 1H, H_{4'}, obscured by solvent peak), 6.33 (s, 1H, H₂), 5.17 (s, 2H, H₁₁), 3.96 (d, $J = 11.6$ Hz, 1H, H_{4B}), 3.81 (s, 3H, NOCH_3), 3.54 (br. s, 3H, NCH_3), 3.16 (d, $J = 11.6$ Hz, 1H, H_{4A}); ^{13}C NMR (CDCl_3 , 101 MHz): δ (C₆, C₈ not shown), 167.2 (C₁₀), 166.3 (C₉), 138.7 (C_{1'}), 134.9 (C_{1a'}), 128.6 (C_{3'}), 128.6 (C_{3a'}),

(2*R*,5*R*)-1-Aza-2-(2-bromopyridin-4-yl)-6-hydroxy-7-(methoxy(methyl)carbamoyl)-5-(4-nitrobenzyloxycarbonyl)-8-oxo-3-thiabicyclo[3.3.0]-oct-6-ene, 106b

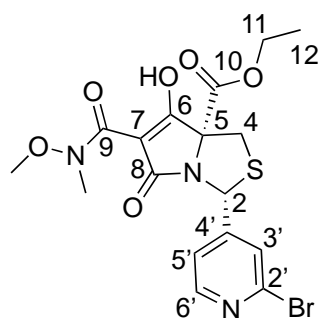


Synthesised from **97b** (1 eqv. 2.3 mmol); yield (550 mg, 41% as inseparable mixture with **107b**, 1 : 1 molar ratio); yellow oil; R_f = 0.31 (EtOAc : MeOH; 9 : 1); ^1H NMR (CDCl_3 , 400 MHz): δ (*OH* not observed), 8.28 (d, J = 5.1 Hz, 1H, H_{6'}), 8.13 (d, J = 8.8 Hz, 2H,

H_{3a'}), 7.63 (s, 1H, H_{3'}), 7.49 (d, J = 5.1 Hz, 1H, H_{5'}), 7.36 (d, J = 8.8 Hz, 2H, H_{2a'}), 6.18 (s, 1H, H₂), 5.29 – 5.18 (m, 2H, H₁₁), 3.87 (dd, J = 11.7, 1H, H_{4B}), 3.80 (s, 3H, NOCH₃), 3.49 (br. s, 3H, NCH₃), 3.16 (d, J = 11.7 Hz, 1H, H_{4A}); ^{13}C NMR (CDCl_3 , 101 MHz): δ (C₆ and C₈ not shown), 166.6 (C₁₀), 166.1 (C₉), 151.4 (C_{6'}), 150.2 (C_{4'}), 147.9 (C_{4a'}), 142.5 (C_{2'}), 141.7 (C_{1a'}), 128.4 (C_{2a'}), 125.8 (C_{3'}), 123.9 (C_{3a'}), 120.9 (C_{5'}), 80.3 (C₇), 66.8 (C₁₁), 63.7 (C₅), 62.5 (C₂ and NOCH₃), 36.2 (NCH₃ and C₄); m/z (ESI⁻) 577 and 579 ([M-H]⁻, 100%); HRMS (ESI⁻) m/z calcd for C₂₂H₁₈O₈N₄BrS [M-H]⁻ 577.0022 and 579.0000; found 577.0034 and 579.0015.

(2*R*,5*R*)-1-Aza-2-(2-bromopyridin-4-yl)-5-ethoxycarbonyl-6-hydroxy-7-

(methoxy(methyl)carbamoyl)-8-oxo-3-thiabicyclo[3.3.0]-oct-6-ene, 107b

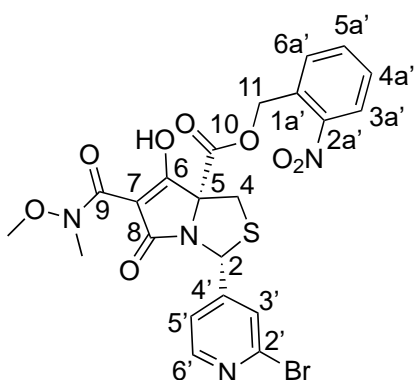


By-product from an inseparable mixture with **106b** or **108b**, 1 : 1 molar ratio; yellow oil; R_f = 0.31 (EtOAc : MeOH; 9 : 1); ^1H NMR (CDCl_3 , 400 MHz): δ (*OH* not observed), 8.21 (d, J = 5.1 Hz, 1H, H_{6'}), 7.59 (s, 1H, H_{3'}), 7.32 – 7.29 (m, 1H, H_{5'}), 6.18 (s, 1H, H_{2'}), 4.22 – 4.14 (m, 2H, H₁₁), 3.85 (dd, J = 11.5, 1H, H_{4B}), 3.79

(s, 3H, NOCH₃), 3.49 (br. s, 3H, NCH₃), 3.11 (d, J = 11.5 Hz, 1H, H_{4A}), 1.19 (t, J = 7.1 Hz, 3H,

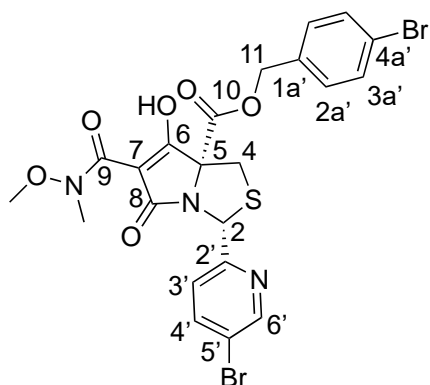
H12); ^{13}C NMR (CDCl_3 , 101 MHz): δ (C6 and C8 not shown), 166.6 (C10), 166.0 (C9), 151.1 (C6'), 150.2 (C4'), 142.4 (C2'), 127.0 (C3'), 120.8 (C5'), 80.1 (C7), 63.3 (C5), 62.4 (C2 and NOCH_3), 61.2 (C11), 36.0 (NCH_3 and C4), 14.0 (C12); m/z (ESI $^-$) 470 and 472 ($[\text{M}-\text{H}]^-$, 100%); $\text{C}_{17}\text{H}_{17}\text{O}_6\text{N}_3\text{BrS}$ ($[\text{M}-\text{H}]^-$).

(2R,5R)-1-Aza-2-(2-bromopyridin-4-yl)-6-hydroxy-7-(methoxy(methyl)carbamoyl)-5-(2-nitrobenzyloxycarbonyl)-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 108b



Synthesised from **98b** (1 eqv. 0.9 mmol); yield (233 mg, 45% as inseparable mixture with **107b**, 1 : 1 molar ratio); yellow oil; R_f = 0.31 (EtOAc : MeOH; 9 : 1); ^1H NMR (CDCl_3 , 400 MHz): δ (OH not observed), 8.31 (d, J = 5.1 Hz, 1H, H6'), 8.06 (d, J = 8.1 Hz, 1H, H3a'), 7.66 (s, 1H, H3'), 7.59 – 7.51 (m, 1H, H5a'), 7.51 – 7.44 (m, 1H, H5'), 7.44 – 7.38 (m, 1H, H4a'), 7.40 – 7.34 (m, 1H, H6a'), 6.19 (s, 1H, H2), 5.61 (d, J = 14.4 Hz, 1H, H11_B), 5.52 (d, J = 14.4 Hz, 1H, H11_A), 3.90 (d, J = 11.7 Hz, 1H, H4_B), 3.83 (s, 3H, NOCH_3), 3.52 (br. s, 3H, NCH_3), 3.18 (d, J = 11.7 Hz, 1H, H4_A); ^{13}C NMR (CDCl_3 , 101 MHz): δ (C6 and C8 not shown), 166.4 (C10), 166.1 (C9), 151.4 (C6'), 150.2 (C4'), 147.5 (C2a'), 142.5 (C2'), 134.1 (C6a'), 133.8 (C1a'), 130.5 (C5a'), 129.4 (C4a'), 125.8 (C3'), 125.2 (C3a'), 120.9 (C5'), 80.3 (C7), 65.3 (C11), 63.3 (C5), 62.6 (C2 and NOCH_3), 36.0 (NCH_3 and C4); m/z (ESI $^-$) 577 and 579 ($[\text{M}-\text{H}]^-$, 100%); HRMS (ESI $^-$) m/z calcd for $\text{C}_{22}\text{H}_{18}\text{O}_8\text{N}_4\text{BrS}$ $[\text{M}-\text{H}]^-$ 577.0022 and 579.0000; found 577.0034 and 579.0015.

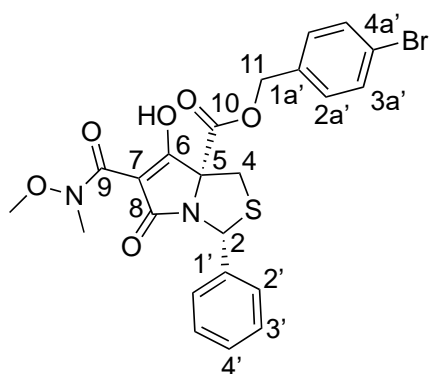
(2*R*,5*R*)-1-Aza-5-(4-bromobenzyloxycarbonyl)-2-(5-bromopyridin-2-yl)-6-hydroxy-7-(methoxy(methyl)carbamoyl)-8-oxo-3-thiabicyclo[3.3.0]-oct-6-ene, 109c



Synthesised from **99c** (1 eqv. 0.3 mmol); yield (65 mg, 35%); pale yellow oil; $R_f = 0.29$ (EtOAc : MeOH; 9 : 1); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ (*OH* not observed), 8.36 (s, 1H, H_{6'}), 7.59 (dd, $J = 8.3, 2.0$ Hz, 1H, H_{4'}), 7.43 (d, $J = 8.3$ Hz, 2H, H_{3a'}), 7.33 (d, $J = 8.3$ Hz, 1H, H_{3'}), 7.12 (d, $J = 8.3$ Hz, 2H, H_{2a'}), 6.31 (s, 1H, H₂), 5.19 (d, $J = 12.4$ Hz, 1H, H_{11B}), 5.08 (d, $J = 12.4$ Hz, 1H, H_{11A}), 3.94 (d, $J = 11.6$ Hz, 1H, H_{4B}), 3.81 (s, 3H, NOCH_3), 3.52 (br. s, 3H, NCH_3), 3.20 (d, $J = 11.6$ Hz, 1H, H_{4A}); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ (C₆ not shown), 177.2 (C₈), 167.1 (C₁₀), 166.3 (C₉), 157.1 (C_{2'}), 150.2 (C_{6'}), 139.4 (C_{4'}), 134.0 (C_{1a'}), 131.8 (C_{3a'}), 130.2 (C_{2a'}), 122.8 (C_{4a'}), 121.6 (C_{3'}), 119.9 (C_{5'}), 81.0 (C₇), 67.5 (C₁₁), 64.8 (C₅), 63.5 (C₂), 63.1 (NOCH_3), 35.9 (C₄), 33.8 (NCH_3); m/z (ESI⁻) 610 (50%), 612 ([M-H]⁻, 100%) and 614 (50%); HRMS (ESI⁻) m/z calcd for $\text{C}_{22}\text{H}_{18}\text{O}_6\text{N}_3\text{Br}_2\text{S}$ [M-H]⁻ 609.9289, 611.9269 and 613.6250; found 609.9273, 611.9252 and 613.9229.

(2*R*,5*R*)-1-Aza-5-(4-bromobenzyloxycarbonyl)-6-hydroxy-7-

(methoxy(methyl)carbamoyl)-8-oxo-2-phenyl-3-thiabicyclo[3.3.0]-oct-6-ene, 109e

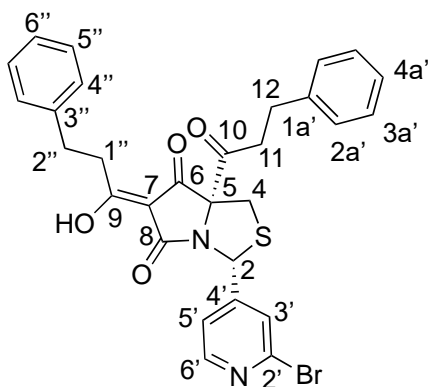


Synthesised from **99e** (1 eqv. 1.7 mmol); yield (496 mg, 56%); purified with column chromatography and wash with 0.5 M HCl solution after column; yellow oil; $R_f = 0.34$ (EtOAc : MeOH; 9 : 1); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ (*OH* not observed), 7.42 – 7.45 (m, 2H, H_{2'}), 7.40 (d, $J = 8.4$ Hz, 2H, H_{3a'}), 7.21 – 7.25 (m, 3H, H_{4'} and H_{3'}), 7.05 (d, $J = 8.5$ Hz, 2H, H_{2a'}), 6.30 (s,

1H, H2), 5.06 (s, 2H, H11), 3.91 (d, $J = 11.6$ Hz, 1H, H4_B), 3.78 (s, 3H, NOCH₃), 3.50 (br. s, 3H, NCH₃), 3.13 (d, $J = 11.6$ Hz, 1H, H4_A); ¹³C NMR (CDCl₃, 101 MHz): δ 185.3 (C6), 177.2 (C8), 167.1 (C10), 166.3 (C9), 138.6 (C1'), 133.9 (C1a'), 131.8 (C3a'), 130.1 (C2a'), 128.5 (C3'), 128.3 (C4'), 126.9 (C2'), 122.6 (C4a'), 80.6 (C7), 67.5 (C11), 64.8 (C5), 62.4 (C2), 61.5 (NOCH₃), 35.7 (C4), 34.4 (NCH₃); m/z (ESI⁻) 531 and 533 ([M-H]⁻, 100%); HRMS (ESI⁻) m/z calcd for C₂₃H₂₀O₆N₂BrS [M-H]⁻ 531.0220 and 533.0200; found 531.0233 and 533.0213.

Tetramate ketones **111-144** were synthesised from the respective tetramate Weinreb amides **101-109** by general procedure **P** (section 6.4.5) with the corresponding Grignard reagent (R²MgBr for the synthesis of **111-143**, R²Mg i Pr for the synthesis of **144**).

(2*R*,5*R*)-1-Aza-2-(2-bromopyridin-4-yl)-7-[(*Z*)-1-hydroxy-3-phenylpropylidene]-6,8-dioxo-5-(3-phenylpropanoyl)dihydro-3-thiabicyclo[3.3.0]-octane, **111b**

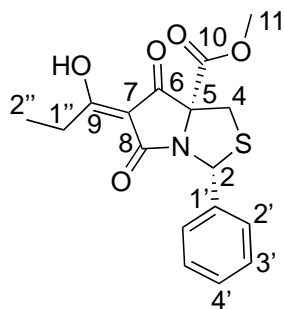


Synthesised from **108b** (1 eqv. 50 mg, 0.1 mmol) with PhCH₂CH₂MgBr; yield (23 mg, 44%); brown oil; $R_f = 0.39$ (100% EtOAc); $[\alpha]_D^{25} = -23.0$ ($c = 1.13$, CHCl₃); $\nu_{\max}/\text{cm}^{-1}$ 1711 (s, C=O), 1654 (s, C=O), 1585 (s, C=C); ¹H NMR (CDCl₃, 400 MHz): δ (OH not observed), 8.34

(d, $J = 5.1$ Hz, 1H, H6'), 7.66 (s, 1H, H3'), 7.31 – 7.12 (m, 10H, H2a', H3a', H4a', H4'', H5'' and H6''), 6.98 (d, $J = 5.1$ Hz, 1H, H5'), 6.27 (s, 1H, H2), 3.91 (d, $J = 11.4$ Hz, 1H, H4_B), 3.26 – 2.55 (m, 9H, H1'', H2'', H11, H12 and H4_A); ¹³C NMR (CDCl₃, 101 MHz): δ 199.6 (C10), 190.8 (C6), 186.9 (C9), 176.1 (C8), 150.7 (C6'), 149.9 (C4'), 143.0 (C2'), 140.1 (C1a'), 139.0 (C3''), 128.8, 128.7, 128.5, 128.4, 128.3, 128.2, 128.1 (C2a', C3a', C4a', C4'', C5''), 126.8 (C6''), 126.5 (C3'), 121.5 (C5'), 100.6 (C7), 87.7 (C5), 61.8 (C2), 38.8 (C11), 34.6 (C4), 34.1

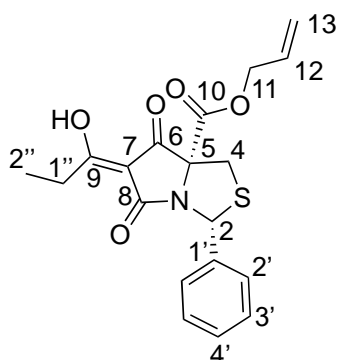
(C1''), 32.0 (C2''), 29.4 (C12); m/z (ESI⁻) 575 and 577 ([M+H]⁻, 100%); HRMS (ESI⁻) m/z calcd for C₂₉H₂₄O₂N₂BrS [M-H]⁻ 575.0635 and 577.0616; found 575.0634 and 575.0614.

(2R,5R)-1-Aza-7-[(E)-1-hydroxypropylidene]-5-methoxycarbonyl-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]-octane, 112e



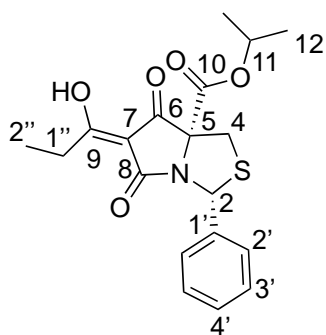
Synthesised from **101e** (1 eqv. 20 mg, 0.05 mmol) with ethylmagnesium bromide; yield (13 mg, 71%); yellow oil; R_f = 0.31 (100% EtOAc); $[\alpha]_D^{25}$ = +272 (c = 1.3, CHCl₃); $\nu_{\max}/\text{cm}^{-1}$ 1751 (s, C=O), 1713 (s, C=O), 1655 (s, C=O), 1598 (s, C=C); ¹H NMR (CDCl₃, 400 MHz): a mixture of 4.1 : 1 AB/CD tautomers: δ (OH not observed), 7.51 (d, J = 7.3 Hz, 2H, H2'), 7.38 – 7.29 (m, 3H, H3' and H4'), 6.47 (s, H2 minor), 6.42 (s, 1H, H2 major), 3.94 (d, J = 11.3 Hz, 1H, H4_B), 3.61 (s, 3H, H11), 3.09 (d, J = 11.3 Hz, 1H, H4_A), 2.88 (q, J = 7.5 Hz, 2H, H1''), 1.22 (d, J = 7.5 Hz, 3H, H2''); ¹³C NMR (CDCl₃, 101 MHz): AB tautomer shown only: δ 192.3 (C6), 187.3 (C9), 175.6 (C8), 167.1 (C10), 138.2 (C1'), 128.6 (C3'), 127.2 (C2' and C4'), 100.0 (C7), 82.1 (C5), 63.7 (C2), 53.5 (C11), 35.4 (C4), 26.7 (C1''), 9.7 (C2''); m/z (ESI⁻) 346 ([M-H]⁻, 100%); HRMS (ESI⁻) m/z calcd for C₁₇H₁₆O₅NS [M-H]⁻ 346.0755; found 346.0758.

(2*R*,5*R*)-5-Allyloxycarbonyl-1-aza-7-[(*E*)-1-hydroxypropylidene]-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]-octane, 113e



Synthesised from **102e** (1 eqv. 23 mg, 0.06 mmol) with ethylmagnesium bromide; yield (10 mg, 45%); yellow oil; $R_f = 0.37$ (100% EtOAc); $[\alpha]_D^{25} = +229$ ($c = 1.0$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1747 (s, C=O), 1714 (s, C=O), 1656 (s, C=O), 1604 (s, C=C); ^1H NMR (CDCl_3 , 400 MHz): a mixture of 3.7 : 1 AB/CD tautomers: δ (OH not observed), 7.52 (d, $J = 6.9$ Hz, 2H, H_{2'}), 7.36 – 7.28 (m, 3H, H_{3'} and H_{4'}), 6.46 (s, H₂ minor), 6.42 (s, 1H, H₂ major), 5.74 – 5.61 (m, 1H, H₁₂), 5.24 – 5.14 (m, 2H, H₁₃), 4.59 – 4.43 (m, 2H, H₁₁), 3.95 (d, $J = 11.3$ Hz, 1H, H_{4B}), 3.09 (d, $J = 11.3$ Hz, 1H, H_{4A}), 2.89 (q, $J = 7.5$ Hz, 2H, H_{1''}), 1.22 (t, $J = 7.5$ Hz, 3H, H_{2''}); ^{13}C NMR (CDCl_3 , 101 MHz): AB tautomer shown only: δ 192.3 (C₆), 187.3 (C₉), 175.8 (C₈), 166.3 (C₁₀), 138.2 (C_{1'}), 131.0 (C₁₂), 128.6, 128.6 (C_{3'} and C_{4'}), 127.2 (C_{2'}), 119.5 (C₁₃), 100.0 (C₇), 82.3 (C₅), 67.4 (C₁₁), 63.8 (C₂), 35.4 (C₄), 26.7 (C_{1''}), 9.7 (C_{2''}); m/z (ESI⁻) 372 ([M-H]⁻, 100%); HRMS (ESI⁻) m/z calcd for C₁₉H₁₈O₅NS [M-H]⁻ 372.0900; found 372.0915.

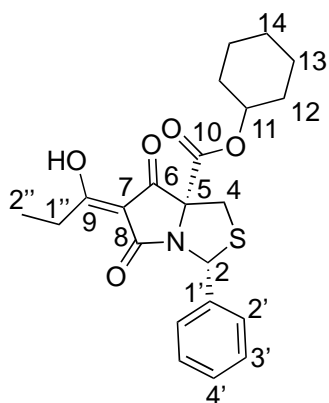
(2*R*,5*R*)-1-Aza-7-[(*E*)-1-hydroxypropylidene]-5-isopropoxycarbonyl-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]-octane, 114e



Synthesised from **103e** (1 eqv. 22 mg, 0.05 mmol) with ethylmagnesium bromide; yield (8 mg, 40%); yellow oil; $R_f = 0.29$ (100% EtOAc); $[\alpha]_D^{25} = +124$ ($c = 0.78$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1747 (s, C=O), 1715 (s, C=O), 1656 (s, C=O), 1608 (s, C=C); ^1H NMR (CDCl_3 , 400 MHz): a mixture of 4.2 : 1 AB/CD tautomers: δ (OH not observed), 7.53 (d, $J = 7.3$ Hz, 2H, H_{2'}), 7.36 – 7.28 (m, 3H, H_{3'} and H_{4'}), 6.46 (s,

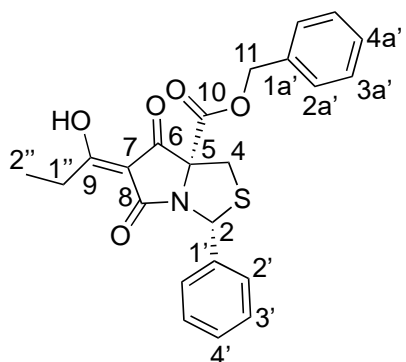
H2 minor), 6.41 (s, 1H, H2), 4.93 (sept, $J = 6.3$ Hz, 1H, H11), 3.94 (d, $J = 11.3$ Hz, 1H, H4_B), 3.06 (d, $J = 11.3$ Hz, 1H, H4_A), 2.93 – 2.83 (m, 2H, H1''), 1.23 (t, $J = 7.6$ Hz, 3H, H2''), 1.13 (d, $J = 6.3$ Hz, 3H, H12_B), 1.00 (d, $J = 6.3$ Hz, 3H, H12_A); ¹³C NMR (CDCl₃, 101 MHz): AB tautomer shown only: δ 192.0 (C6), 187.6 (C9), 175.9 (C8), 166.0 (C10), 138.4 (C1'), 128.6 (C3'), 128.5 (C4'), 127.2 (C2'), 100.1 (C7), 82.4 (C5), 71.1 (C11), 63.7 (C2), 35.3 (C4), 26.7 (C1''), 21.5 (C12), 21.3 (C12), 9.8 (C2''); m/z (ESI⁻) 374 ([M-H]⁻, 100%); HRMS (ESI⁻) m/z calcd for C₁₉H₂₀O₅NS [M-H]⁻ 374.1068; found 374.1071.

(2*R*,5*R*)-1-Aza-5-cyclohexyloxycarbonyl-7-[(*E*)-1-hydroxypropylidene]-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]-octane, 115e



Synthesised from **104e** (1 eqv. 43 mg, 0.1 mmol) with ethylmagnesium bromide; yield (32 mg, 80%); yellow oil; $R_f = 0.33$ (100% EtOAc); $[\alpha]_D^{25} = +208$ ($c = 0.90$, CHCl₃); $\nu_{\max}/\text{cm}^{-1}$ 1745 (s, C=O), 1713 (s, C=O), 1654 (s, C=O), 1601 (s, C=C); ¹H NMR (CDCl₃, 400 MHz): a mixture of 4.5 : 1 AB/CD tautomers: δ (OH not observed), 7.53 (d, $J = 7.1$ Hz, 2H, H2'), 7.36 – 7.26 (m, 3H, H3' and H4'), 6.44 (s, H2 minor), 6.40 (s, 1H, H2 major), 4.79 – 4.68 (m, 1H, H11), 3.93 (d, $J = 11.3$ Hz, 1H, H4_B), 3.07 (d, $J = 11.3$ Hz, 1H, H4_A), 2.95 – 2.81 (m, 2H, H1''), 1.74 – 1.67 (m, 2H, H12), 1.62 – 1.54 (m, 4H, H12, H13), 1.49 – 1.41 (m, 2H, H13), 1.37 – 1.27 (m, 2H, H14), 1.22 (t, $J = 7.5$ Hz, 3H, H2''); ¹³C NMR (CDCl₃, 101 MHz): AB tautomer shown only: δ 191.9 (C6), 187.7 (C9), 175.8 (C8), 165.8 (C10), 138.4 (C1'), 128.6 (C3'), 128.5 (C4'), 127.2 (C2'), 100.1 (C7), 82.5 (C5), 75.6 (C11), 63.7 (C2), 35.1 (C4), 31.0, 30.9 (C12), 26.6 (C1''), 25.3 (C14), 23.4, 23.4 (C13), 9.8 (C2''); m/z (ESI⁻) 414 ([M-H]⁻, 100%); HRMS (ESI⁻) m/z calcd for C₂₂H₂₄O₅NS [M-H]⁻ 414.1381; found 414.1389.

(2*R*,5*R*)-1-Aza-5-benzyloxycarbonyl-7-[(*E*)-1-hydroxypropylidene]-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]-octane, 116e



Synthesised from **105e** (1 eqv. 35 mg, 0.08 mmol) with ethylmagnesium bromide; yield (14 mg, 43%); yellow oil;

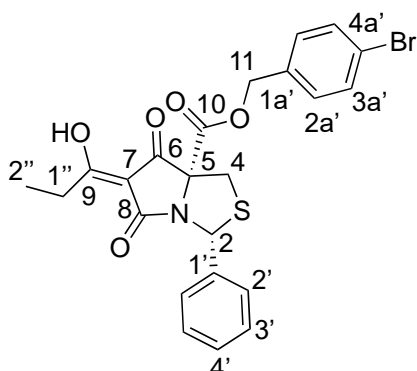
$R_f = 0.41$ (100% EtOAc); $[\alpha]_D^{25} = +75.6$ ($c = 0.40$, CHCl_3);

$\nu_{\text{max}}/\text{cm}^{-1}$ 1746 (s, C=O), 1714 (s, C=O), 1657 (s, C=O),

1602 (s, C=C); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): a mixture of 4.2 :

1 AB/CD tautomers: δ (OH not observed), 7.47 (m, 2H, H_2'), 7.30 – 7.25 (m, 6H, obscured by solvent, H_3' , $\text{H}_3\text{a}'$, H_4' and $\text{H}_4\text{a}'$), 7.14 (dd, $J = 6.4, 2.8$ Hz, 2H, $\text{H}_2\text{a}'$), 6.44 (s, H_2 minor) 6.40 (s, 1H, H_2 major), 5.12 (d, $J = 12.3$ Hz, 1H, $\text{H}_{11\text{A}}$), 5.00 (d, $J = 12.3$ Hz, 1H, $\text{H}_{11\text{B}}$), 3.96 (d, $J = 11.4$ Hz, 1H, $\text{H}_{4\text{B}}$), 3.10 (d, $J = 11.4$ Hz, 1H, $\text{H}_{4\text{A}}$), 2.87 (q, $J = 7.6$ Hz, 2H, $\text{H}_{1''}$), 1.21 (t, $J = 7.6$ Hz, 3H, H_2''); $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 151 MHz): AB tautomer shown only: δ 192.3 (C6), 187.3 (C9), 175.9 (C8), 166.6 (C10), 138.2 (C1'), 134.7 (C1a'), 128.7, 128.7 (C3' and C3a'), 128.6, 128.6, 128.4 (C2a', C4', C4a'), 127.2 (C2'), 100.1 (C7), 82.4 (C5), 68.6 (C11), 64.0 (C2), 35.4 (C5), 26.7 (C1''), 9.7 (C2''); m/z (ESI⁻) 422 ($[\text{M}-\text{H}]^-$, 100%); HRMS (ESI⁻) m/z calcd for $\text{C}_{23}\text{H}_{20}\text{O}_5\text{NS}$ $[\text{M}-\text{H}]^-$ 422.1068; found 422.1075.

(2*R*,5*R*)-1-Aza-5-(4-bromobenzoyloxycarbonyl)-7-[(*E*)-1-hydroxypropylidene]-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]-octane, 117e



Synthesised from **109e** (1 eqv. 46 mg, 0.09 mmol) with ethylmagnesium bromide; yield (18 mg, 42%); yellow oil;

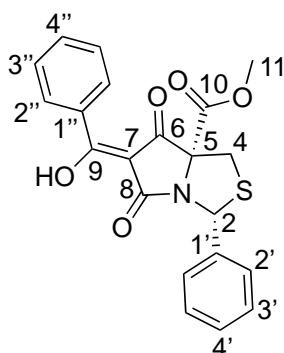
$R_f = 0.40$ (100% EtOAc); $[\alpha]_D^{25} = +169$ ($c = 0.80$, CHCl_3);

$\nu_{\text{max}}/\text{cm}^{-1}$ 1749 (s, C=O), 1713 (s, C=O), 1655 (s, C=O),

1600 (s, C=C); $^1\text{H NMR}$ (CDCl_3 , 600 MHz): a mixture of

3.3 : 1 AB/CD tautomers: δ (*OH* not observed), 7.49 – 7.42 (m, 2H, H2'), 7.40 (d, $J = 8.4$ Hz, 2H, H3a'), 7.29 – 7.21 (m, obscured by solvent, 3H, H3' and H4'), 6.97 (d, $J = 8.4$ Hz, 2H, H2a'), 6.42 (s, H2 minor), 6.40 (s, 1H, H2 major), 5.02 (d, $J = 12.4$ Hz, 1H, H11_B), 4.96 (d, $J = 12.4$ Hz, 1H, H11_A), 3.95 (d, $J = 11.4$ Hz, 1H, H4_B), 3.10 (d, $J = 11.4$ Hz, 1H, H4_A), 2.87 (q, $J = 7.6$ Hz, 2H, H1''), 1.22 (t, $J = 7.6$ Hz, 3H, H2''); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): AB tautomer shown only: δ 192.5 (C6), 187.3 (C9), 175.9 (C8), 166.6 (C10), 138.1 (C1'), 133.8 (C1a'), 131.9 (C3a'), 130.1 (C2a'), 128.7 (C3'), 128.6 (C4'), 127.2 (C2'), 122.8 (C4a'), 98.5 (C7), 82.3 (C5), 67.7 (C11), 63.9 (C2), 35.4 (C4), 26.7 (C1''), 9.7 (C2''); m/z (ESI⁻) 500 and 502 ([M-H]⁻, 100%); HRMS (ESI⁻) m/z calcd for $\text{C}_{23}\text{H}_{19}\text{O}_5\text{NBrS}$ [M-H]⁻ 500.0173 and 502.0153; found 500.0180 and 502.0159.

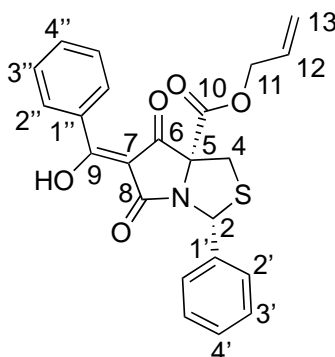
(2*R*,5*R*)-1-Aza-7-[(*Z*)-hydroxyl(phenyl)methylene]-5-methoxycarbonyl-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]-octane, 118e



Synthesised from **101e** (1 eqv. 30 mg, 0.08 mmol) with phenylmagnesium bromide; yield (10 mg, 33%); pale yellow oil; $R_f = 0.43$ (100% EtOAc); $[\alpha]_D^{25} = +186$ ($c = 0.90$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1750 (s, C=O), 1709 (s, C=O), 1652 (s, C=O), 1591 (s, C=C), 1560 (s, C=C); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ (*OH* not observed), 8.18 (d, $J =$

7.5 Hz, 2H, H2''), 7.64 (t, $J = 7.5$ Hz, 1H, H4''), 7.53 (app dt, $J = 15.6, 7.5$ Hz, 4H, H2' and H3''), 7.35 (app dt, $J = 15.4, 7.1$ Hz, 3H, H4' and H3'), 6.49 (s, 1H, H2), 3.98 (d, $J = 11.4$ Hz, 1H, H4_B), 3.65 (s, 3H, H11), 3.14 (d, $J = 11.4$ Hz, 1H, H4_A); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ 185.9 (C6), 182.9 (C9), 177.7 (C8), 167.1 (C10), 138.1 (C1'), 134.8 (C1''), 130.1 (C3''), 128.7 (C3'), 128.4 (C4' and C4''), 127.2 (C2' and C2''), 96.8 (C7), 81.5 (C5), 64.2 (C2), 53.6 (C11), 35.7 (C4); m/z (ESI⁺) 418 ([M+Na]⁺, 100%); HRMS (ESI⁺) m/z calcd for $\text{C}_{21}\text{H}_{17}\text{O}_5\text{NNaS}$ [M+Na]⁺ 418.0720; found 418.0720.

(2*R*,5*R*)-5-Allyloxycarbonyl-1-aza-7-[(*Z*)-hydroxyl(phenyl)methylene]-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]-octane, 119e

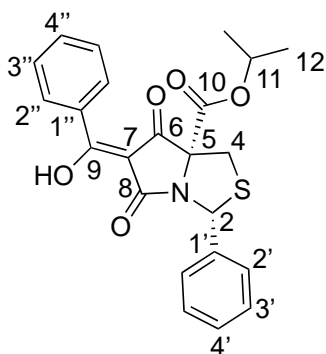


Synthesised from **102e** (1 eqv. 21 mg, 0.05 mmol) with phenylmagnesium bromide; yield (10 mg, 46%); yellow oil; $R_f = 0.50$ (100% EtOAc); $[\alpha]_D^{25} = +2.04$ ($c = 1.0$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1750 (s, C=O), 1708 (s, C=O), 1650 (s, C=O), 1591 (s, C=C), 1559 (s, C=C); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): a mixture of 0.2 : 1

AB/CD tautomers: δ (*OH* not observed), 8.17 (d, $J = 7.9$ Hz, 2H, H2''), 7.64 (t, $J = 7.5$ Hz, 1H, H4''), 7.53 (app dt, $J = 15.6, 7.4$ Hz, 4H, H2' and H3''), 7.34 (app dt, $J = 10.9, 6.8$ Hz, 3H, H4'

and H3'), 6.49 (s, 1H, H2 major), 6.40 (s, H2 minor), 5.62 – 5.79 (m, 1H, H12), 5.14 – 5.32 (m, 2H, H13), 4.47 – 4.64 (m, 2H, H11), 3.99 (d, $J = 11.4$ Hz, 1H, H4_B), 3.14 (d, $J = 11.4$ Hz, 1H, H4_A); ¹³C NMR (CDCl₃, 101 MHz): a mixture of AB/CD tautomers: δ 189.9 (C6 AB), 185.9 (C6 CD), 182.9 (C9 CD), 181.5 (C6 AB), 177.9 (C8 CD), 177.1 (C8 AB), 166.6, 166.4 (C10), 139.7, 138.1 (C1'), 134.8, 134.4 (C1''), 131.0 (C12), 130.2, 130.0 (C3''), 129.0 (C3'), 128.7 (C4''), 128.4 (C4'), 127.2 (C2'), 126.6 (C2''), 118.4 (C13), 100.5 (C7 AB), 99.0 (C7 CD), 81.6 (C5), 70.0, 67.5 (C11), 64.4, 62.9 (C2), 35.7, 33.8 (C4); m/z (ESI⁺) 444 ([M+Na]⁺, 100%); HRMS (ESI⁺) m/z calcd for C₂₃H₁₉O₅NNaS [M+Na]⁺ 444.0876; found 444.0876.

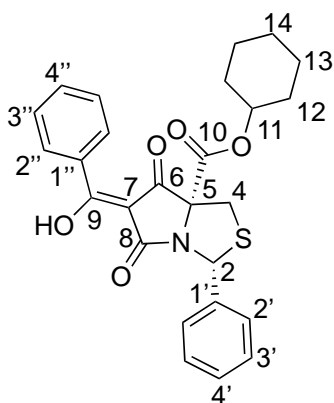
(2R,5R)-1-Aza-7-[(Z)-hydroxyl(phenyl)methylene]-5-isopropoxycarbonyl-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]-octane, 120e



Synthesised from **103e** (1 eqv. 50 mg, 0.1 mmol) with phenylmagnesium bromide; yield (32 mg, 62%); pale yellow oil; $R_f = 0.56$ (100% EtOAc); $[\alpha]_D^{25} = +235$ ($c = 0.93$, CHCl₃); $\nu_{\max}/\text{cm}^{-1}$ 1745 (s, C=O), 1709 (s, C=O), 1652 (s, C=O), 1592 (s, C=C), 1560 (s, C=C); ¹H NMR (CDCl₃, 400 MHz): δ (OH not observed),

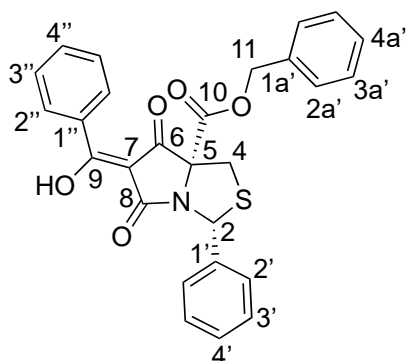
8.17 (d, $J = 7.4$ Hz, 2H, H2''), 7.64 (t, $J = 7.4$ Hz, 1H, H4''), 7.58 (d, $J = 7.2$ Hz, 2H, H2'), 7.51 (t, $J = 7.4$ Hz, 2H, H3''), 7.34 (app dt, $J = 13.4, 7.2$ Hz, 3H, H4' and H3'), 6.49 (s, 1H, H2), 4.98 (sept, $J = 6.3$ Hz, 1H, H11), 3.98 (d, $J = 11.3$ Hz, 1H, H4_B), 3.11 (d, $J = 11.3$ Hz, 1H, H4_A), 1.17 (d, $J = 6.3$ Hz, 3H, H12_B), 1.03 (d, $J = 6.3$ Hz, 3H, H12_A); ¹³C NMR (CDCl₃, 101 MHz): δ 186.2 (C6), 182.6 (C9), 177.9 (C8), 166.0 (C10), 138.3 (C1'), 134.7 (C1''), 130.1 (C3''), 128.6 (C3'), 128.4 (C4' and C4''), 127.2 (C2' and C2''), 99.0 (C7), 81.7 (C5), 71.2 (C11), 64.2 (C2), 35.5 (C4), 21.6 (C12_B), 21.3 (C12_A); m/z (ESI⁺) 424 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for C₂₃H₂₂O₅NS [M+H]⁺ 424.1213; found 424.1213.

(2*R*,5*R*)-1-Aza-5-cyclohexyloxycarbonyl-7-[(*Z*)-hydroxyl(phenyl)methylene]-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]-octane, 121e



Synthesised from **104e** (1 eqv. 55 mg, 0.1 mmol) with phenylmagnesium bromide; yield (30 mg, 53%); yellow oil; $R_f = 0.54$ (100% EtOAc); $[\alpha]_D^{25} = +180$ ($c = 0.90$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1746 (s, C=O), 1709 (s, C=O), 1653 (s, C=O), 1593 (s, C=C), 1560 (s, C=C); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): a 0.1 : 1 mixture of AB/CD tautomers: δ (OH not observed), 8.16 (d, $J = 7.4$ Hz, 2H, H2''), 7.64 (t, $J = 7.4$ Hz, 1H, H4''), 7.57 (d, $J = 7.2$ Hz, 2H, H2'), 7.51 (t, $J = 7.4$ Hz, 2H, H3''), 7.34 (app dt, $J = 12.6, 6.9$ Hz, 3H, H3' and H4'), 6.50 (s, H2 minor tautomer), 6.48 (s, 1H, H2 major tautomer), 4.72 – 4.83 (m, 1H, H11), 3.98 (d, $J = 11.4$ Hz, 1H, H4_B), 3.12 (d, $J = 11.4$ Hz, 1H, H4_A), 1.55 – 1.68 (m, 4H, H12), 1.16 – 1.43 (m, 6H, H13 and H14); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ 186.4 (C6), 182.5 (C9), 177.9 (C8), 166.0 (C10), 138.3 (C1'), 134.7 (C1''), 131.0, 130.1 (C3''), 128.6 (C3'), 128.6 (C4''), 128.4 (C4'), 127.2 (C2' and C2''), 99.1 (C7), 81.9 (C5), 75.8 (C11), 64.3 (C2), 35.4 (C4), 31.1 (C12), 31.0 (C12), 25.3 (C14), 23.5 (C13), 23.5 (C13); m/z (ESI⁻) 462 ([M-H]⁻, 100%); HRMS (ESI⁻) m/z calcd for $\text{C}_{26}\text{H}_{24}\text{O}_5\text{NS}$ [M-H]⁻ 462.1381; found 462.1383.

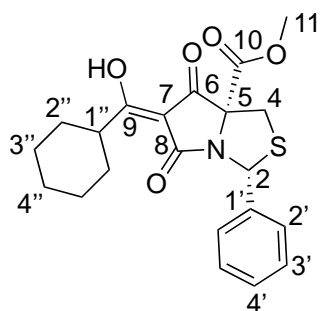
(2*R*,5*R*)-1-Aza-5-benzyloxycarbonyl-7-[(*Z*)-hydroxyl(phenyl)methylene]-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]-octane, 122e



Synthesised from **105e** (1 eqv. 42 mg, 0.09 mmol) with phenylmagnesium bromide; yield (13 mg, 30%); orange oil; $R_f = 0.50$ (100% EtOAc); $[\alpha]_D^{25} = +1.69$ ($c = 1.3$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1747 (s, C=O), 1708 (s, C=O), 1651 (s, C=O), 1591 (s, C=C), 1557 (s, C=C); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ

(OH not observed), 8.13 (d, $J = 7.5$ Hz, 2H, H2''), 7.64 (t, $J = 7.5$ Hz, 1H, H4''), 7.47 – 7.52 (m, 4H, H2' and H3''), 7.27 – 7.32 (m, 6H, H3', H3a', H4' and H4a'), 7.18 (dd, $J = 6.6, 2.9$ Hz, 2H, H2a'), 6.47 (s, 1H, H2), 5.17 (d, $J = 12.2$ Hz, 1H, H11_B), 5.05 (d, $J = 12.2$ Hz, 1H, H11_A), 4.00 (d, $J = 11.5$ Hz, 1H, H4_B), 3.15 (d, $J = 11.5$ Hz, 1H, H4_A); $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 126 MHz): δ 185.9 (C6), 182.9 (C9), 178.0 (C8), 166.7 (C10), 138.0 (C1'), 134.8 (C1a'), 134.7 (C1''), 130.2 (C3''), 128.7 (C3' and C3a'), 128.6, 128.5, 128.4 (C2'', C2a', C4', C4a', C4''), 127.2 (C2'), 99.0 (C7), 81.8 (C5), 68.7 (C11), 64.6 (C2), 35.7 (C4); m/z (ESI⁺) 472 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for $\text{C}_{27}\text{H}_{22}\text{O}_5\text{NS}$ [M+H]⁺ 472.1213; found 472.1214.

(2*R*,5*R*)-1-Aza-7-[(*E*)-cyclohexyl(hydroxy)methylene]-5-methoxycarbonyl-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]-octane, 123e

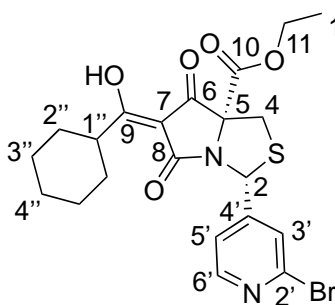


Synthesised from **101e** (1 eqv. 35 mg, 0.09 mmol) with cyclohexylmagnesium bromide; yield (13 mg, 35%); yellow oil; $R_f = 0.29$ (petrol: EtOAc; 7 : 3); $[\alpha]_D^{25} = +155$ ($c = 1.1$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1751 (s, C=O), 1713 (s, C=O), 1653 (s, C=O), 1598 (s,

C=C); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): a 3.8 : 1 mixture of AB/CD tautomers: δ (OH not observed), 7.51 (d, $J = 7.5$ Hz, 2H, H2'), 7.33 (app dt, $J = 14.4, 7.0$ Hz, 3H, H4' and H3'),

6.44 (s, H2 minor tautomer), 6.41 (s, 1H, H2 major tautomer), 3.94 (d, $J = 11.3$ Hz, 1H, H4_B), 3.64 (s, H11 minor tautomer), 3.62 (s, 3H, H11 major tautomer), 3.30 – 3.39 (m, 1H, H1''), 3.08 (d, $J = 11.3$ Hz, 1H, H4_A), 1.69 – 1.86 (m, 6H, H3'' and H2''), 1.45 – 1.59 (m, 2H, H3''), 1.30 – 1.44 (m, 2H, H4''); ¹³C NMR (CDCl₃, 101 MHz): δ 195.3 (C6), 187.1 (C9), 182.4 (C9 minor tautomer), 176.1 (C8), 167.1 (C10), 138.3 (C1'), 128.6 (C3' and C4'), 127.2 (C2'), 99.0 (C7), 82.1 (C5), 63.7 (C2), 53.5 (C11), 41.5 (C1''), 35.5 (C4), 28.9 (C2''), 28.8 (C2''), 25.6 (C3''), 25.6 (C3''), 25.5 (C4''); m/z (ESI⁻) 400 ([M-H]⁻, 100%); HRMS (ESI⁻) m/z calcd for C₂₁H₂₂O₅NS [M-H]⁻ 400.1224; found 400.1227.

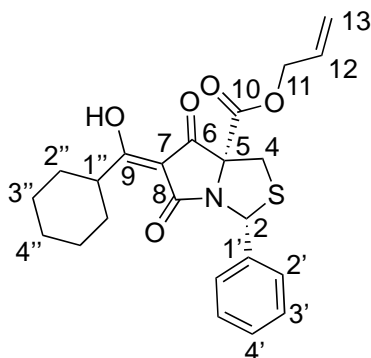
(2*R*,5*R*)-1-Aza-2-(2-bromopyridin-4-yl)-7-[(*E*)-cyclohexyl(hydroxy)methylene]-5-ethoxycarbonyl-6,8-dioxo-3-thiabicyclo[3.3.0]-octane, 124b



12 Synthesised from **107b** (1 eqv. 32 mg, 0.07 mmol) with cyclohexylmagnesium bromide; yield (11 mg, 33%); brown oil; $R_f = 0.26$ (petrol ether : EtOAc; 1 : 4); $[\alpha]_D^{25} = +149$ ($c = 1.06$, CHCl₃); $\nu_{\max}/\text{cm}^{-1}$ 1748 (s, C=O), 1715 (s, C=O), 1656 (s, C=O), 1586 (s, C=C); ¹H NMR (CDCl₃, 400 MHz): a mixture of 3.3 : 1 AB/CD tautomers: δ (OH not observed), 8.34 (d, $J = 5.1$ Hz, 1H, H6'), 7.66 (s, 1H, H3'), 7.38 (d, $J = 5.1$ Hz, 1H, H5'), 6.30 (s, H2 minor), 6.28 (s, 1H, H2 major), 4.16 (q, $J = 7.1$ Hz, 2H, H11), 3.93 (d, $J = 11.4$ Hz, 1H, H4_B), 3.40 – 3.32 (m, 1H, H1''), 3.09 (d, $J = 11.4$ Hz, 1H, H4_A), 1.88 – 1.72 (m, 6H, H3'' and H2''), 1.59 – 1.46 (m, 2H, H3''), 1.43 – 1.31 (m, 2H, H4''), 1.17 (t, $J = 7.1$ Hz, 3H, H12); ¹³C NMR (CDCl₃, 101 MHz): δ 195.9 (C6), 186.3 (C9), 176.4 (C8), 166.1 (C10), 150.7 (C6'), 150.4 (C4'), 142.7 (C2'), 125.9 (C3'), 121.1 (C5'), 98.6 (C7), 82.3 (C5), 63.7 (C2 minor), 63.5 (C2 major), 62.2 (C11 minor), 61.7 (C11 major), 41.5 (C1''), 35.9 (C4), 28.9 (C2''), 28.8 (C2''), 25.6 (C3''), 25.5 (C3''), 25.5 (C4''), 14.0 (C12); m/z (ESI⁻) 493 and 495

([M-H]⁻, 100%); HRMS (ESI⁻) *m/z* calcd for C₂₁H₂₂O₅N₂BrS [M-H]⁻ 493.0438 and 495.0418; found 493.0430 and 493.0407.

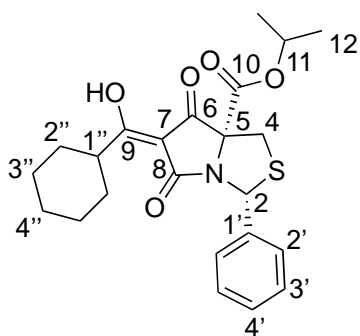
(2*R*,5*R*)-5-Allyloxycarbonyl-1-aza-7-[(*E*)-cyclohexyl(hydroxy)methylene]-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]-octane, 125e



Synthesised from **102e** (1 eqv. 24 mg, 0.06 mmol) with cyclohexylmagnesium bromide; yield (10 mg, 39%); pale yellow oil; *R_f* = 0.42 (100% EtOAc); [α]_D²⁵ = +11.0 (*c* = 0.70, CHCl₃); ν_{max} /cm⁻¹ 1751 (s, C=O), 1715 (s, C=O), 1654 (s, C=O), 1599 (s, C=C); ¹H NMR (CDCl₃, 400 MHz): a mixture of 4.0 :

1 AB/CD tautomers: δ (*OH* not observed), 7.52 (d, *J* = 6.4 Hz, 2H, H_{2'}), 7.28 – 7.35 (m, 3H, H_{3'} and H_{4'}), 6.44 (s, H₂ minor tautomer), 6.41 (s, 1H, H₂ major tautomer), 5.61 – 5.74 (m, 1H, H₁₂), 5.14 – 5.25 (m, 2H, H₁₃), 4.45 – 4.59 (m, 2H, H₁₁), 3.95 (d, *J* = 11.3 Hz, 1H, H_{4_B}), 3.29 – 3.42 (m, 1H, H_{1''}), 3.08 (d, *J* = 11.3 Hz, 1H, H_{4_A}), 1.70 – 1.86 (m, 6H, H_{3''} and H_{2''}), 1.45 – 1.59 (m, 2H, H_{3''}), 1.32 – 1.44 (m, 2H, H_{4''}); ¹³C{¹H} NMR (CDCl₃, 126 MHz): δ 195.3 (C₆), 187.1 (C₉), 176.3 (C₈), 166.4 (C₁₀), 138.3 (C_{1'}), 131.0 (C₁₂), 128.6 (C_{3'}), 128.6 (C_{4'}), 127.2 (C_{2'}), 119.5 (C₁₃), 99.0 (C₇), 82.3 (C₅), 67.4 (C₁₁), 63.8 (C₂), 41.4 (C_{1''}), 35.5 (C₄), 28.9 (C_{2''}), 28.8 (C_{2''}), 25.6 (C_{3''}), 25.6 (C_{3''}), 25.5 (C_{4''}); *m/z* (ESI⁻) 426 ([M-H]⁻, 100%); HRMS (ESI⁻) *m/z* calcd for C₂₃H₂₄O₅NS [M-H]⁻ 426.1381; found 426.1385.

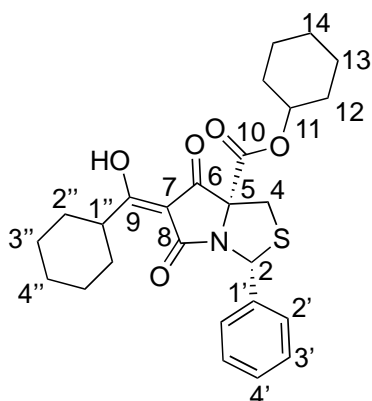
(2*R*,5*R*)-1-Aza-7-[(*E*)-cyclohexyl(hydroxy)methylene]-5-isopropoxycarbonyl-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]-octane, 126e



Synthesised from **103e** (1 eqv. 32 mg, 0.08 mmol) with cyclohexylmagnesium bromide; yield (10 mg, 30%); yellow oil; $R_f = 0.26$ (petrol ether : EtOAc; 3 : 7); $[\alpha]_D^{25} = +163$ ($c = 0.60$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1748 (s, C=O), 1714 (s, C=O), 1655 (s, C=O), 1601 (s, C=C); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): a mixture of

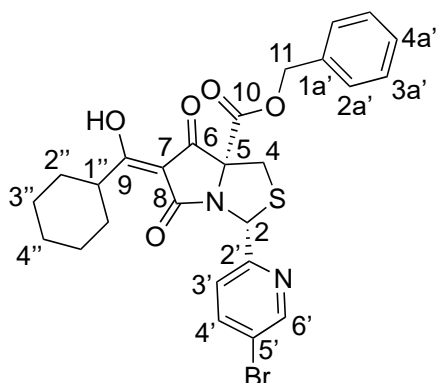
4.0 : 1 AB/CD tautomers: δ (OH not observed), 7.53 (d, $J = 7.3$ Hz, 2H, H_2'), 7.28 – 7.36 (m, 3H, H_3' and H_4'), 6.43 (s, H_2 minor tautomer), 6.40 (s, 1H, H_2 major tautomer), 4.94 (sept, $J = 6.3$ Hz, 1H, H_{11}), 3.93 (d, $J = 11.3$ Hz, 1H, $\text{H}_{4\text{B}}$), 3.36 (tt, $J = 11.6, 3.2$ Hz, 1H, $\text{H}_{1''}$), 3.06 (d, $J = 11.3$ Hz, 1H, $\text{H}_{4\text{A}}$), 1.68 – 1.88 (m, 6H, $\text{H}_{3''}$ and $\text{H}_{2''}$), 1.45 – 1.60 (m, 2H, $\text{H}_{3''}$), 1.31 – 1.45 (m, 2H, $\text{H}_{4''}$), 1.14 (d, $J = 6.3$ Hz, 3H, $\text{H}_{12\text{B}}$), 1.01 (d, $J = 6.3$ Hz, 3H, $\text{H}_{12\text{A}}$); $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 126 MHz): δ 195.1 (C6), 187.4 (C9), 176.4 (C8), 166.0 (C10), 138.5 (C1'), 128.6 (C3'), 128.5 (C4'), 127.2 (C2'), 99.0 (C7), 82.4 (C5), 71.1 (C11), 63.7 (C2), 41.4 (C1''), 35.4 (C4), 28.9 (C2''), 28.7 (C2''), 25.6 (C3''), 25.6 (C3''), 25.5 (C4''), 21.5 (C12_B), 21.3 (C12_A); m/z (ESI⁻) 428 ($[\text{M}-\text{H}]^-$, 100%); HRMS (ESI⁻) m/z calcd for $\text{C}_{23}\text{H}_{26}\text{O}_5\text{NS}$ $[\text{M}-\text{H}]^-$ 428.1537; found 428.1539.

(2*R*,5*R*)-1-Aza-5-cyclohexyloxycarbonyl-7-[(*E*)-cyclohexyl(hydroxy)methylene]-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]-octane, 127e



Synthesised from **104e** (1 eqv. 30 mg, 0.07 mmol) with cyclohexylmagnesium bromide; yield (18 mg, 57%); pale yellow oil; $R_f = 0.47$ (petrol ether : EtOAc; 7 : 3); $[\alpha]_D^{25} = +3.12$ ($c = 1.0$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1745 (s, C=O), 1713 (s, C=O), 1655 (s, C=O), 1600 (s, C=C); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): a mixture of 4.3 : 1 AB/CD tautomers: δ (OH not observed), 7.53 (d, $J = 6.3$ Hz, 2H, H2'), 7.28 – 7.35 (m, 3H, H3' and H4'), 6.42 (s, H2 minor tautomer), 6.39 (s, 1H, H2 major tautomer), 4.68 – 4.80 (m, 1H, H11), 3.93 (d, $J = 11.3$ Hz, 1H, H4_B), 3.36 (tt, $J = 11.5, 3.1$ Hz, 1H, H1''), 3.06 (d, $J = 11.3$ Hz, 1H, H4_A), 1.65 – 1.89 (m, 8H, H12 and H2''), 1.40 – 1.65 (m, 8H, H3'' and H13), 1.32 – 1.39 (m, 4H, H4'' and H14); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ 195.0 (C6), 187.6 (C9), 176.4 (C8), 165.9 (C10 major tautomer), 164.7 (C10 minor tautomer), 138.5 (C1'), 128.6 (C3'), 128.5 (C4'), 127.2 (C2'), 99.1 (C7), 82.5 (C5), 75.6 (C11), 63.8 (C2 major tautomer), 62.3 (C2 minor tautomer), 41.3 (C1''), 35.2 (C4), 31.1 (C12), 31.0 (C12), 28.9 (C2''), 28.7 (C2''), 25.6 (C3''), 25.6 (C3''), 25.5 (C4''), 25.3 (C14), 23.5 (C13), 23.5 (C13); m/z (ESI⁺) 492 ([M+Na]⁺, 100%); HRMS (ESI⁺) m/z calcd for $\text{C}_{26}\text{H}_{31}\text{O}_5\text{NNaS}$ [M+Na]⁺ 492.1815; found 492.1816.

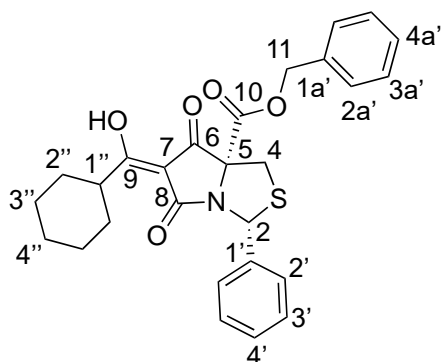
(2*R*,5*R*)-1-Aza-5-benzyloxycarbonyl-2-(5-bromopyridin-2-yl)-7-[(*E*)-cyclohexyl(hydroxy)methylene]-6,8-dioxo-3-thiabicyclo[3.3.0]-octane, 128c



Synthesised from **105c** (1 eqv. 50 mg, 0.09 mmol) with cyclohexylmagnesium bromide; yield (15 mg, 29%); orange oil; $R_f = 0.32$ (100% EtOAc); $[\alpha]_D^{25} = +41.0$ ($c = 0.99$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1745 (s, C=O), 1712 (s, C=O), 1655 (s, C=O), 1598 (s, C=C); $^1\text{H NMR}$ (CDCl_3 , 400 MHz):

a mixture of 3.7 : 1 AB/CD tautomers: δ (OH not observed), 8.56 (d, $J = 2.0$ Hz, 1H, H6'), 7.84 – 7.76 (m, 1H, H4'), 7.37 – 7.28 (m, 3H, H3a' and H4a'), 7.24 – 7.15 (m, 3H, H2a' and H3'), 6.38 (s, H2 minor tautomer), 6.35 (s, 1H, H2 major tautomer), 5.21 (d, $J = 12.3$ Hz, 1H, H11_B), 5.11 (d, $J = 12.3$ Hz, 1H, H11_A), 3.94 (d, $J = 11.5$ Hz, 1H, H4_B), 3.39 – 3.34 (m, 1H, H1''), 3.14 (d, $J = 11.5$ Hz, 1H, H4_A), 1.86 – 1.70 (m, 6H, H3'' and H2''), 1.55 – 1.46 (m, 2H, H3''), 1.42 – 1.35 (m, 2H, H4''); $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 126 MHz): δ 195.7 (C6 AB), 194.3 (C6 CD), 187.0 (C9 AB), 186.0 (C9 CD), 176.8 (C8 AB), 172.9 (C8 CD), 166.8 (C10 CD), 166.5 (C10 AB), 156.5, 156.4 (C2'), 151.3, 150.4 (C6'), 139.5, 139.4 (C4'), 134.7 (C1a'), 128.7 (C3a'), 128.6 (C4a'), 127.1 (C2a'), 122.9, 121.6 (C3'), 120.2, 120.0 (C5'), 102.0 (C7 CD), 98.9 (C7 AB), 85.1, 83.3 (C5), 68.6 (C11), 64.3 (C2), 41.5, 41.3 (C1''), 35.8 (C4), 28.9, 28.9, 28.8, 28.7 (C2''), 25.7, 25.6, 25.6, 25.5, 25.5, 25.5 (C3'' and C4''); m/z (ESI⁻) 555 and 557 ([M-H]⁻, 100%); HRMS (ESI⁻) m/z calcd for $\text{C}_{26}\text{H}_{24}\text{O}_5\text{N}_2\text{BrS}$ [M-H]⁻ 555.0595 and 557.0574; found 555.0590 And 557.0578.

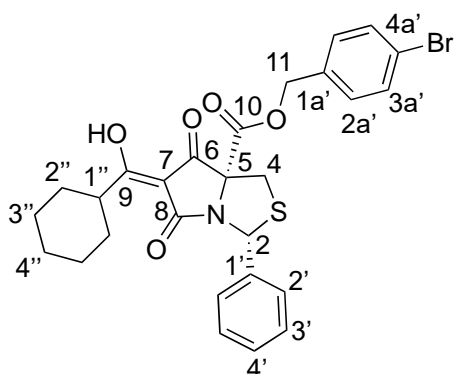
(2*R*,5*R*)-1-Aza-5-benzyloxycarbonyl-7-[(*E*)-cyclohexyl(hydroxy)methylene]-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]-octane, 128e



Synthesised from **105e** (1 eqv. 72 mg, 0.2 mmol) with cyclohexylmagnesium bromide; yield (28 mg, 37%); yellow oil; $R_f = 0.67$ (100% EtOAc); $[\alpha]_D^{25} = +45.1$ ($c = 0.60$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1749 (s, C=O), 1712 (s, C=O), 1656 (s, C=O), 1597 (s, C=C); $^1\text{H NMR}$ (CDCl_3 , 400

MHz): a mixture of 4.6 : 1 AB/CD tautomers: δ (OH not observed), 7.49 – 7.44 (m, 2H, H2'), 7.32 – 7.27 (m, 3H, H3' and H4'), 7.26 – 7.23 (m, 3H, H3a' and H4a'), 7.17 – 7.12 (m, 2H, H2a'), 6.42 (s, H2 minor tautomer), 6.38 (s, 1H, H2 major tautomer), 5.14 (d, $J = 12.3$ Hz, 1H, H11_B), 5.00 (d, $J = 12.3$ Hz, 1H, H11_A), 3.96 (d, $J = 11.4$ Hz, 1H, H4_B), 3.34 (tt, $J = 11.6$, 3.2 Hz, 1H, H1''), 3.10 (d, $J = 11.4$ Hz, 1H, H4_A), 1.88 – 1.68 (m, 6H, H3'' and H2''), 1.60 – 1.46 (m, 2H, H3''), 1.45 – 1.30 (m, 2H, H4''); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): AB major: δ 195.3 (C6), 187.1 (C9), 176.4 (C8), 166.6 (C10), 138.2 (C1'), 134.7 (C1a'), 128.7, 128.6, 128.6, 128.5 (C3' and C3a'), 128.4, 128.3 (C4' and C4a'), 127.2, 127.1 (C2' and C2a'), 99.0 (C7), 82.4 (C5), 68.5 (C11), 64.0 (C2), 41.4 (C1''), 35.4 (C4), 28.9 (C2''), 28.7 (C2''), 25.6 (C3''), 25.6 (C3''), 25.5 (C4''); m/z (ESI⁺) 478 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for $\text{C}_{27}\text{H}_{28}\text{O}_5\text{NS}$ [M+H]⁺ 478.1683; found 478.1682.

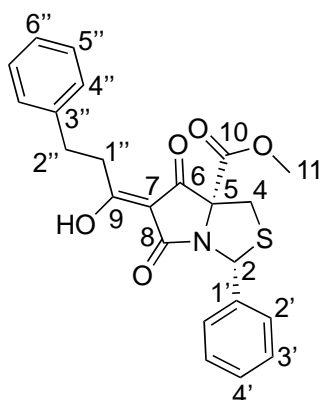
(2*R*,5*R*)-1-Aza-5-(4-bromobenzoyloxycarbonyl)-7-[(*E*)-cyclohexyl(hydroxy)methylene]-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]-octane, 129e



Synthesised from **109e** (1 eqv. 37 mg, 0.07 mmol) with cyclohexylmagnesium bromide; yield (14 mg, 36%); yellow oil; $R_f = 0.68$ (100% EtOAc); $[\alpha]_D^{25} = +66.0$ ($c = 0.90$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1750 (s, C=O), 1712 (s, C=O), 1655 (s, C=O), 1595 (s, C=C); $^1\text{H NMR}$ (CDCl_3 ,

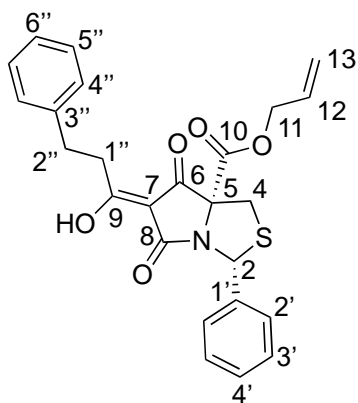
400 MHz): a mixture of 5.1 : 1 AB/CD tautomers: δ (OH not observed), 7.48 – 7.41 (m, 3H, H2' and H4'), 7.39 (d, $J = 8.4$ Hz, 2H, H3a'), 7.28 – 7.22 (m, 2H, obscured by solvent peak, H3'), 6.97 (d, $J = 8.4$ Hz, 2H, H2a'), 6.41 (s, H2 minor tautomer), 6.38 (s, 1H, H2 major tautomer), 5.08 – 4.91 (m, 2H, H11) 3.93 (d, $J = 11.4$ Hz, 1H, H4_B), 3.32 (tt, $J = 11.6, 3.3$ Hz, 1H, H1''), 3.08 (d, $J = 11.4$ Hz, 1H, H4_A), 1.87 – 1.69 (m, 6H, H3'' and H2''), 1.57 – 1.44 (m, 2H, H3'), 1.43 – 1.29 (m, 2H, H4''); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ 195.5 (C6 AB), 186.9 (C9 AB), 176.4 (C8 AB), 166.6 (C10), 138.2 (C1'), 133.7 (C1a'), 131.9 (C3a' minor), 131.8 (C3a' major), 130.1 (C2a' major), 130.0 (C2a' minor), 128.6 (C3'), 128.6 (C4'), 127.2 (C2' minor), 127.1 (C2' major), 122.7 (C4a'), 98.9 (C7), 82.3 (C5), 67.8 (C11 minor), 67.6 (C11 major), 63.9 (C2), 41.5 (C1''), 35.4 (C4), 28.9 (C2''), 28.7 (C2''), 25.6 (C3''), 25.5 (C4''); m/z (ESI⁻) 554 and 556 ([M-H]⁻, 100%); HRMS (ESI⁻) m/z calcd for $\text{C}_{27}\text{H}_{25}\text{O}_5\text{NBrS}$ [M-H]⁻ 554.0642 and 556.0623; found 554.0265 and 556.0628.

(2*R*,5*R*)-1-Aza-7-[(*Z*)-1-hydroxy-3-phenylpropylidene]-5-methoxycarbonyl-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]-octane, 130e



Synthesised from **101e** (1 eqv. 40 mg, 0.1 mmol) with phenethylmagnesium bromide; yield (18 mg, 40%); pale yellow oil; $R_f = 0.37$ (100% EtOAc); $[\alpha]_D^{25} = +148$ ($c = 0.90$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1752 (s, C=O), 1715 (s, C=O), 1655 (s, C=O), 1601 (s, C=C); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ (*OH* not observed), 7.50 (d, $J = 7.7$ Hz, 2H, H_{2'}), 7.19 – 7.38 (m, 8H, H_{4'}, H_{3'}, H_{4''}, H_{5''} and H_{6''}), 6.40 (s, 1H, H₂), 3.91 (d, $J = 11.3$ Hz, 1H, H_{4B}), 3.60 (s, 3H, H₁₁), 3.10 – 3.24 (m, 2H, H_{2''}), 2.99 (d, $J = 8.1$ Hz, 2H, H_{1''}), 2.98 (d, $J = 11.3$ Hz, 1H, H_{4A}); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ 189.8 (C₆), 187.1 (C₉), 175.3 (C₈), 167.0 (C₁₀), 139.4 (C_{3''}), 138.2 (C_{1'}), 128.7 (C_{3'} and C_{5''}), 128.6, 128.6 (C_{4'} and C_{4''}), 127.2 (C_{2'}), 126.7 (C_{6''}), 100.8 (C₇), 82.1 (C₅), 63.7 (C₂), 53.5 (C₁₁), 35.3 (C₄), 34.7 (C_{1''}), 32.0 (C_{2''}); m/z (ESI⁺) 424 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for C₂₃H₂₂O₅NS [M+H]⁺ 424.1213; found 424.1215.

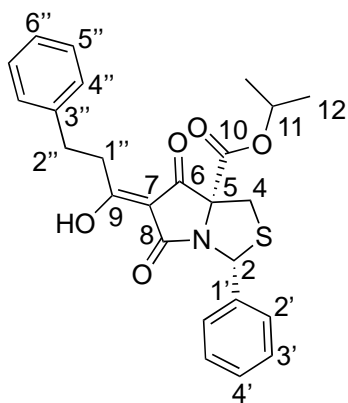
(2*R*,5*R*)-5-Allyloxycarbonyl-1-aza-7-[(*Z*)-1-hydroxy-3-phenylpropylidene]-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]-octane, 131e



Synthesised from **102e** (1 eqv. 27 mg, 0.07 mmol) with phenethylmagnesium bromide; yield (10 mg, 33%); yellow oil; $R_f = 0.54$ (100% EtOAc); $[\alpha]_D^{25} = +2.91$ ($c = 1.0$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1711 (s, C=O), 1652 (s, C=O), 1600 (s, C=C); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): a mixture of 0.8 : 1 AB/CD tautomers: δ (*OH* not observed), 7.55 (d, $J = 7.2$ Hz, 2H, H_{2'} minor tautomer), 7.51 (d, $J = 6.9$ Hz, 2H, H_{2'} major tautomer), 7.17 – 7.40 (m, 16H, H_{4'},

H3', H4'', H5'' and H6''), 6.41 (s, 1H, H2 minor, AB), 6.40 (s, 1H, H2 major, CD), 5.58 – 5.73 (m, 2H, H12), 5.10 – 5.26 (m, 4H, H13), 4.41 – 4.58 (m, 4H, H11), 4.08 (d, $J = 11.4$ Hz, 1H, H4_B minor tautomer), 3.92 (d, $J = 11.4$ Hz, 1H, H4_B major tautomer), 3.08 – 3.34 (m, 4H, H2''), 2.85 – 3.04 (m, 6H, H1'' and H4_A); $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 126 MHz): δ 191.5 (C6 AB), 189.8 (C6 CD), 188.1 (C9 AB), 187.2 (C9 CD), 175.5 (C8 CD), 174.7 (C8 AB), 166.2 (C10), 140.4, 139.4 (C3''), 138.1, 137.8 (C1'), 130.9 (C12), 129.0, 128.7 (C3'), 128.6, 128.6 (C5''), 128.6, 128.5 (C4''), 128.3, 128.1 (C4'), 127.2, 126.7 (C2'), 126.5, 126.3 (C6''), 119.5 (C13), 102.3 (C7 AB), 100.8 (C7 CD), 87.3, 82.2 (C5), 70.1, 67.4 (C11), 64.2, 63.3 (C2), 35.3 (C4), 34.7, 34.5 (C1''), 32.1, 32.0 (C2''); m/z (ESI⁺) 472 ([M+Na]⁺, 100%); HRMS (ESI⁺) m/z calcd for $\text{C}_{25}\text{H}_{23}\text{O}_5\text{NNaS}$ [M+Na]⁺ 472.1189; found 472.1193.

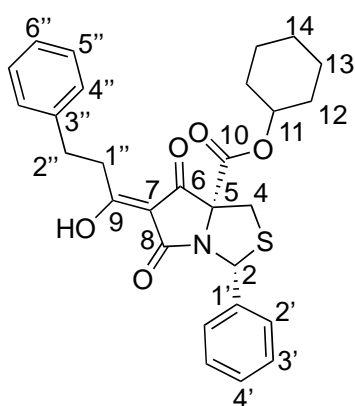
(2*R*,5*R*)-1-Aza-7-[(*Z*)-1-hydroxy-3-phenylpropylidene]-5-isopropoxycarbonyl-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]-octane, 132e



Synthesised from **103e** (1 eqv. 74 mg, 0.2 mmol) with phenethylmagnesium bromide; yield (37 mg, 45%); pale yellow oil; $R_f = 0.44$ (100% EtOAc); $[\alpha]_D^{25} = +153$ ($c = 1.1$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1747 (s, C=O), 1713 (s, C=O), 1653 (s, C=O), 1600 (s, C=C); ^1H NMR (CDCl_3 , 400 MHz): a mixture of 0.1 : 1 AB/CD tautomers: δ (OH not observed), 7.53 (d, $J = 7.0$ Hz, 2H, H2'), 7.17 – 7.38 (m, 8H, H4', H3', H4'', H5'' and H6''), 6.41 (s, H2 minor tautomer), 6.40 (s, 1H, H2 major tautomer), 4.87 – 4.98 (m, 1H, H11), 4.08 (d, $J = 11.3$ Hz, H4_B minor tautomer), 3.91 (d, $J = 11.3$ Hz, 1H, H4_B, major tautomer), 3.08 – 3.28 (m, 2H, H2''), 2.97 – 3.09 (m, 2H, H1''), 2.96 (d, $J = 11.3$ Hz, 1H, H4_A), 1.12 (d, $J = 6.3$ Hz, 3H, H12_B), 0.99 (d, $J = 6.3$ Hz, 3H, H12_A); ^{13}C NMR (CDCl_3 , 101 MHz): CD major only: δ 189.5 (C6), 187.5 (C9),

175.6 (C8), 165.9 (C10), 139.4 (C3''), 138.3 (C1'), 128.7 (C3' and C5''), 128.6, 128.5 (C4' and C4''), 127.2 (C2'), 126.7 (C6''), 100.8 (C7), 82.4 (C5), 71.1 (C11), 63.6 (C2), 35.2 (C4), 34.7 (C1''), 32.0 (C2''), 21.5 (C12_B), 21.2 (C12_A); *m/z* (ESI⁺) 452 ([M+H]⁺, 100%); HRMS (ESI⁺) *m/z* calcd for C₂₅H₂₆O₅NS [M+H]⁺ 452.1526; found 452.1527.

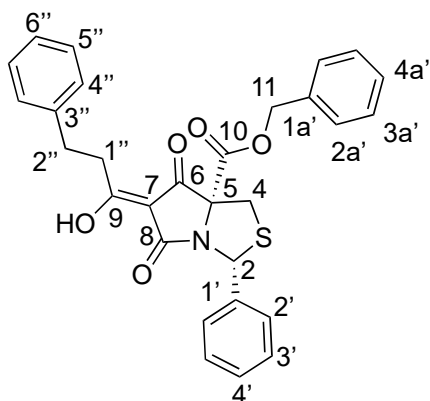
(2*R*,5*R*)-1-Aza-5-cyclohexyloxycarbonyl-7-[(*Z*)-1-hydroxy-3-phenylpropylidene]-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]-octane, 133e



Synthesised from **104e** (1 eqv. 55 mg, 0.1 mmol) with phenethylmagnesium bromide; yield (44 mg, 73%); yellow oil; *R_f* = 0.47 (100% EtOAc); $[\alpha]_{\text{D}}^{25} = +114$ (*c* = 0.98, CHCl₃); $\nu_{\text{max}}/\text{cm}^{-1}$ 1746 (s, C=O), 1714 (s, C=O), 1654 (s, C=O), 1602 (s, C=C); ¹H NMR (CDCl₃, 400 MHz): a mixture of 0.2 : 1 AB/CD tautomers: δ (*OH* not observed), 7.54 (d, *J* = 7.0 Hz, 2H, H2'),

7.19 – 7.38 (m, 8H, H4', H3', H4'', H5'' and H6''), 6.42 (s, H2 minor tautomer), 6.40 (s, 1H, H2 major tautomer), 4.68 – 4.78 (m, 1H, H11), 4.09 (d, *J* = 11.1 Hz, H4_B minor tautomer), 3.92 (d, *J* = 11.3 Hz, 1H, H4_B major tautomer), 3.13 – 3.24 (m, 2H, H2''), 2.95 – 3.05 (m, 2H, H1''), 2.99 (d, *J* = 11.3 Hz, 1H, H4_A), 1.54 – 1.65 (m, 4H, H12), 1.16 – 1.38 (m, 6H, H14 and H13); ¹³C NMR (CDCl₃, 101 MHz): CD major only: δ 189.4 (C6), 187.6 (C9), 175.6 (C8), 165.8 (C10), 139.5 (C3''), 138.3 (C1'), 128.7 (C3' and C5''), 128.6, 128.5 (C4' and C4''), 127.2 (C2'), 126.7 (C6''), 100.9 (C7), 82.5 (C5), 75.7 (C11), 63.7 (C2), 35.1 (C4), 34.7 (C1''), 32.0 (C2''), 31.1 (C12), 30.9 (C12), 25.3 (C14), 23.5 (C13); *m/z* (ESI⁻) 490 ([M-H]⁻, 100%); HRMS (ESI⁻) *m/z* calcd for C₂₈H₂₈O₅NS [M-H]⁻ 490.1694; found 490.1697.

(2*R*,5*R*)-1-Aza-5-benzyloxycarbonyl-7-[(*Z*)-1-hydroxy-3-phenylpropylidene]-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]-octane, 134e



Synthesised from **105e** (1 eqv. 27 mg, 0.06 mmol) with phenethylmagnesium bromide; yield (12 mg, 40%);

pale yellow oil; $R_f = 0.51$ (100% EtOAc); $[\alpha]_D^{25} = +1.46$

($c = 1.2$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1749 (s, C=O), 1713 (s, C=O),

1654 (s, C=O), 1599 (s, C=C); $^1\text{H NMR}$ (CDCl_3 , 400 MHz):

δ (OH not observed), 7.46 (dd, $J = 6.4, 2.9$ Hz, 2H, H2'),

7.21 – 7.31 (m, 11H, H4', H4a', H3', H3a', H4'', H5'' and H6''), 7.13 (dd, $J = 6.4, 2.9$ Hz, 2H,

H2a'), 6.38 (s, 1H, H2), 5.11 (d, $J = 12.2$ Hz, 1H, H11_B), 4.99 (d, $J = 12.2$ Hz, 1H, H11_A), 3.93

(d, $J = 11.4$ Hz, 1H, H4_B), 3.11 – 3.19 (m, 2H, H2''), 3.00 (d, $J = 11.4$ Hz, 1H, H4_A), 2.93 –

2.98 (m, 2H, H1''); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ 189.8 (C6), 187.1 (C9), 175.6 (C8), 166.5

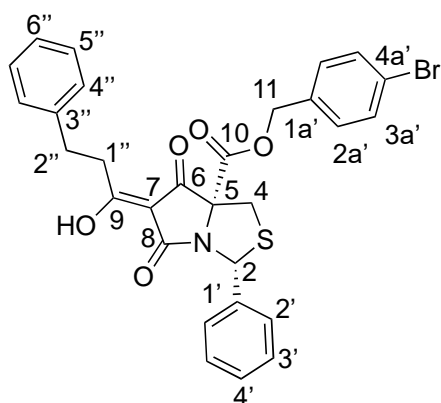
(C10), 139.4 (C3''), 138.1 (C1'), 134.6 (C1a'), 128.7, 128.5, 128.4 (C2a', C3', C3a', C4', C4a',

C4'', C5''), 127.2 (C2'), 126.7 (C6''), 100.8 (C7), 82.4 (C5), 68.6 (C11), 63.9 (C2), 35.3 (C4),

34.8 (C1''), 32.0 (C2''); m/z (ESI⁺) 500 ($[\text{M}+\text{H}]^+$, 100%); HRMS (ESI⁺) m/z calcd for

$\text{C}_{29}\text{H}_{26}\text{O}_5\text{NS}$ $[\text{M}+\text{H}]^+$ 500.1526; found 500.1525.

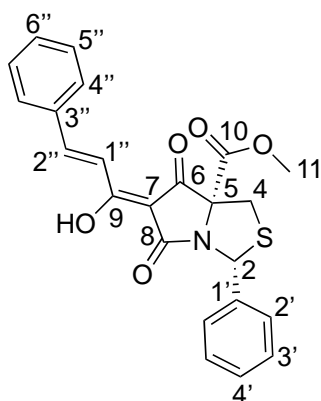
(2*R*,5*R*)-1-Aza-5-(4-bromobenzoyloxycarbonyl)-7-[(*Z*)-1-hydroxy-3-phenylpropylidene]-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]-octane, 135e



Synthesised from **109e** (1 eqv. 30 mg, 0.06 mmol) with phenethylmagnesium bromide; yield (10 mg, 31%); pale yellow oil; $R_f = 0.50$ (100% EtOAc); $[\alpha]_D^{25} = +54.0$ ($c = 0.50$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1749 (s, C=O), 1713 (s, C=O), 1627 (s, C=O), 1599 (s, C=C); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): a mixture of 0.1 : 1 AB/CD tautomers: δ

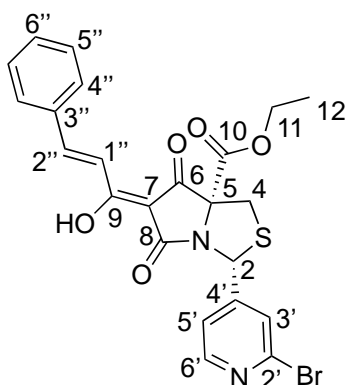
(OH not observed), 7.47 – 7.41 (m, 2H, H2'), 7.39 (d, $J = 8.4$ Hz, 2H, H3a'), 7.30 – 7.19 (m, 8H, H4', H3', H4'', H5'' and H6''), 6.96 (d, $J = 8.4$ Hz, 2H, H2a'), 6.43 (s, H2 minor, AB), 6.38 (s, 1H, H2 major, CD), 5.00 (d, $J = 12.4$ Hz, 1H, H11_B), 4.94 (d, $J = 12.4$ Hz, 1H, H11_A), 3.91 (d, $J = 11.4$ Hz, 1H, H4_B), 3.22 – 3.11 (m, 2H, H2''), 3.00 – 2.94 (m, 3H, H4_A and H1''); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): CD major only: δ 190.1 (C6), 187.1 (C9), 175.6 (C8), 166.5 (C10), 139.5 (C3''), 138.1 (C1'), 133.4 (C1a'), 132.0 (C3a'), 130.2 (C2a'), 128.8, 128.8, 128.6 (C3', C4', C4'', C5''), 127.3 (C2'), 126.8 (C6''), 122.9 (C4a'), 100.8 (C7), 82.4 (C5), 67.8 (C11), 63.9 (C2), 35.4 (C4), 34.9 (C1''), 32.1 (C2''); m/z (ESI⁻) 576 and 578 ([M-H]⁻, 100%); HRMS (ESI⁻) m/z calcd for $\text{C}_{29}\text{H}_{23}\text{O}_5\text{NBrS}$ [M-H]⁻ 576.0475 and 578.0456; found 576.0492 and 578.0474.

(2*R*,5*R*)-1-Aza-7-[(*Z*,2*E*)-1-hydroxy-3-phenylallylidene]-5-methoxycarbonyl-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]-octane, 136e



Synthesised from **101e** (1 eqv. 40 mg, 0.1 mmol) with β -styrylmagnesium bromide; yield (26 mg, 58%); yellow oil; $R_f = 0.51$ (100% EtOAc); $[\alpha]_D^{25} = +214$ ($c = 0.95$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1750 (s, C=O), 1706 (s, C=O), 1652 (s, C=O), 1625 (s, C=C), 1581 (s, C=C), 1563 (s, C=C); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): a 0.26 : 1 mixture of AB/CD tautomers: δ (*OH* not observed), 7.96 (d, $J = 16.0$ Hz, 1H, H1''), 7.73 (d, $J = 16.0$ Hz, 1H, H2''), 7.66 (dd, $J = 7.6, 1.9$ Hz, 2H, H4''), 7.54 (d, $J = 7.5$ Hz, 2H, H2'), 7.28 – 7.47 (m, 6H, H3', H4', H5'' and H6''), 6.51 (s, H2 minor tautomer), 6.46 (s, 1H, H2 major tautomer), 3.98 (d, $J = 11.3$ Hz, 1H, H4_B), 3.64 (s, 3H, H11), 3.14 (d, $J = 11.3$ Hz, 1H, H4_A); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): CD major only: δ 187.5 (C6), 176.2 (C9), 176.0 (C8), 167.2 (C10), 146.9 (C2''), 138.4 (C1'), 134.3 (C3''), 131.9 (C4''), 129.4, 129.3 (C3' and C5''), 128.6, 128.6 (C4' and C6''), 127.2 (C2'), 117.4 (C1''), 99.5 (C7), 82.2 (C5), 63.9 (C2), 53.5 (C11), 35.5 (C4); m/z (ESI⁻) 420 ([M-H]⁻, 100%); HRMS (ESI⁻) m/z calcd for $\text{C}_{23}\text{H}_{18}\text{O}_5\text{NS}$ [M-H]⁻ 420.0911; found 420.0914.

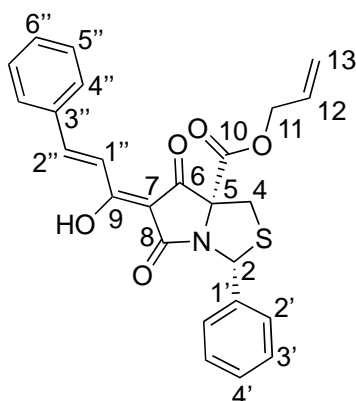
(2*R*,5*R*)-1-Aza-2-(2-bromopyridin-4-yl)-5-ethoxycarbonyl-7-[(*Z*,2*E*)-1-hydroxy-3-phenylallylidene]-6,8-dioxo-thiabicyclo[3.3.0]-octane, **137b**



Synthesised from **107b** (1 eqv. 98 mg, 0.2 mmol) with β -styrylmagnesium bromide; yield (33 mg, 31%); orange oil; R_f = 0.16 (petrol ether : EtOAc; 3 : 7); $[\alpha]_D^{25} = +89.6$ ($c = 1.11$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1747 (s, C=O), 1708 (s, C=O), 1653 (s, C=O), 1622 (s, C=C), 1580 (s, C=C), 1561 (s, C=C); $^1\text{H NMR}$ (CDCl_3 ,

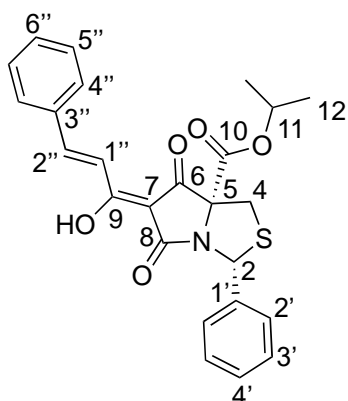
400 MHz): a 0.34 : 1 mixture of AB/CD tautomers: δ (OH not observed), 8.35 (d, $J = 5.0$ Hz, 1H, H6'), 7.99 (d, $J = 16.0$ Hz, 1H, H1''), 7.73 (d, $J = 16.0$ Hz, 1H, H2''), 7.70 – 7.61 (m, 3H, H3' and H4''), 7.50 – 7.35 (m, 4H, H5', H5'' and H6''), 6.36 (s, H2 minor tautomer), 6.32 (s, 1H, H2 major tautomer), 4.17 (q, $J = 7.1$ Hz, 2H, H11), 3.96 (d, $J = 11.4$ Hz, 1H, H4_B), 3.19 (d, $J = 11.5$ Hz, H4_A minor), 3.15 (d, $J = 11.4$ Hz, 1H, H4_A major), 1.18 (t, $J = 7.1$ Hz, 3H, H12); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): major CD tautomer only: δ 186.7 (C6), 176.6 (C9), 176.3 (C8), 166.2 (C10), 150.7 (C6'), 150.4 (C4'), 147.7 (C2''), 142.7 (C2'), 134.1 (C3''), 132.2 (C4''), 129.6, 129.3 (C5''), 128.5 (C6''), 125.9 (C3'), 121.1 (C5'), 117.1 (C1''), 99.0 (C7), 82.4 (C5), 63.5 (C2), 61.8 (C11), 35.9 (C4), 14.0 (C12); m/z (ESI⁻) 513 and 515 ($[\text{M}-\text{H}]^-$, 100%); HRMS (ESI⁻) m/z calcd for $\text{C}_{23}\text{H}_{18}\text{O}_5\text{N}_2\text{BrS}$ $[\text{M}-\text{H}]^-$ 513.0125 and 515.0105; found 513.0116 and 515.0093.

(2*R*,5*R*)-5-Allyloxycarbonyl-1-aza-7-[(*Z*,*E*)-1-hydroxy-3-phenylallylidene]-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]-octane, 138e



Synthesised from **102e** (1 eqv. 22 mg, 0.05 mmol) with β -styrylmagnesium bromide; yield (14 mg, 58%); yellow oil; R_f = 0.32 (petrol ether : EtOAc; 3 : 7); $[\alpha]_D^{25} = +6.83$ ($c = 1.0$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1749 (s, C=O), 1706 (s, C=O), 1652 (s, C=O), 1625 (s, C=C), 1581 (s, C=C), 1564 (s, C=C); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): a 0.17 : 1 mixture of AB/CD tautomers: δ (OH not observed), 7.96 (d, $J = 15.9$ Hz, 1H, H1''), 7.73 (d, $J = 15.9$ Hz, 1H, H2''), 7.67 (dd, $J = 7.5$, 1.9 Hz, 2H, H4''), 7.55 (d, $J = 7.0$ Hz, 2H, H2'), 7.31 – 7.47 (m, 6H, H3', H4', H5'' and H6''), 6.51 (s, H2 minor tautomer), 6.46 (s, 1H, H2 major tautomer), 5.63 – 5.75 (m, 1H, H12), 5.15 – 5.26 (m, 2H, H13), 4.49 – 4.56 (m, 2H, H11), 3.99 (d, $J = 11.4$ Hz, 1H, H4_B), 3.14 (d, $J = 11.4$ Hz, 1H, H4_A); $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 126 MHz): major CD tautomer: δ 187.5 (C6), 176.2 (C9), 176.1 (C8), 166.5 (C10), 146.9 (C2''), 138.3 (C1'), 134.2 (C3''), 131.9 (C4''), 131.0 (C12), 129.5, 129.3 (C3' and C5''), 128.6, 128.6 (C4' and C6''), 127.2 (C2'), 119.6 (C13), 117.4 (C1''), 99.5 (C7), 82.3 (C5), 67.5 (C11), 64.0 (C2), 35.5 (C4); minor AB tautomer: δ 196.3 (C6), 176.7 (C9), 175.0 (C8), 169.0 (C10), 146.0 (C2''), 139.9 (C1'), 134.4 (C3''), 131.6 (C4''), 130.9 (C12), 129.6, 129.2 (C3' and C5''), 128.6, 128.5 (C4' and C6''), 126.6 (C2'), 119.6 (C13), 117.6 (C1''), 101.1 (C7), 80.6 (C5), 70.5 (C11), 62.5 (C2), 35.7 (C4); m/z (ESI⁻) 446 ($[\text{M}-\text{H}]^-$, 100%); HRMS (ESI⁻) m/z calcd for $\text{C}_{25}\text{H}_{20}\text{O}_5\text{NS}$ $[\text{M}-\text{H}]^-$ 446.1068; found 446.1070.

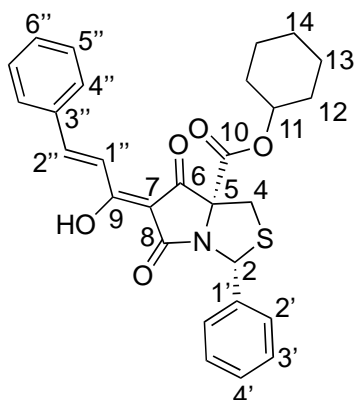
(2*R*,5*R*)-1-Aza-7-[(*Z*,*E*)-1-hydroxy-3-phenylallylidene]-5-isopropoxy carbonyl-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]-octane, 139e



Synthesised from **103e** (1 eqv. 40 mg, 0.1 mmol) with β -styrylmagnesium bromide; yield (34 mg, 77%); yellow oil; R_f = 0.34 (petrol ether : EtOAc; 3 : 7); $[\alpha]_D^{25} = +287$ ($c = 1.0$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1744 (s, C=O), 1707 (s, C=O), 1652 (s, C=O), 1625 (s, C=C), 1581 (s, C=C), 1565 (s, C=C); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): a 0.28 : 1 mixture of AB/CD tautomers: δ (OH not observed),

7.96 (d, $J = 16.0$ Hz, 1H, H1''), 7.74 (d, $J = 16.0$ Hz, 1H, H2''), 7.67 (dd, $J = 7.4, 1.9$ Hz, 2H, H4''), 7.57 (d, $J = 7.2$ Hz, 2H, H2'), 7.29 – 7.47 (m, 6H, H3', H4', H5'' and H6''), 6.50 (s, H2 minor tautomer), 6.46 (s, 1H, H2 major tautomer), 4.95 (sept, $J = 6.2$ Hz, 1H, H11), 3.97 (d, $J = 11.3$ Hz, 1H, H4_B), 3.11 (d, $J = 11.3$ Hz, 1H, H4_A), 1.16 (d, $J = 6.2$ Hz, 3H, H12_B), 1.02 (d, $J = 6.2$ Hz, 3H, H12_A); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): major CD tautomer: δ 187.7 (C6), 176.3 (C9), 176.0 (C8), 166.1 (C10), 146.7 (C2''), 138.5 (C1'), 134.3 (C3''), 131.9 (C4''), 129.4, 129.2 (C3' and C5''), 128.6, 128.5 (C4' and C6''), 127.3 (C2'), 117.5 (C1''), 99.6 (C7), 82.5 (C5), 71.2 (C11), 63.8 (C2), 35.5 (C4), 21.5 (C12_B), 21.3 (C12_A); minor AB tautomer: δ 196.7 (C6), 176.5 (C9), (C8 not shown), 169.1 (C10), 147.3 (C2''), 139.1 (C1'), 134.3 (C3''), 132.0 (C4''), 129.5, 129.3 (C3' and C5''), 128.6, 128.3 (C4' and C6''), 127.3 (C2'), 116.7 (C1''), 101.8 (C7), 80.8 (C5), 71.3 (C11), 64.1 (C2), 35.6 (C4), 21.5 (C12_B), 21.3 (C12_A); m/z (ESI⁺) 472 ([M+Na]⁺, 100%); HRMS (ESI⁺) m/z calcd for $\text{C}_{25}\text{H}_{23}\text{O}_5\text{NNaS}$ [M+Na]⁺ 472.1189; found 472.1190.

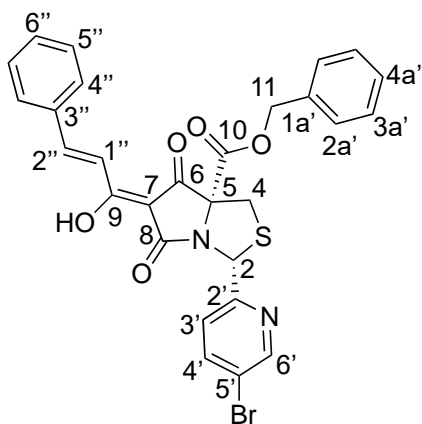
(2*R*,5*R*)-1-Aza-5-cyclohexyloxycarbonyl-7-[(*Z*,2*E*)-1-hydroxy-3-phenylallylidene]-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]-octane, **140e**



Synthesised from **104e** (1 eqv. 57 mg, 0.1 mmol) with β -styrylmagnesium bromide; yield (33 mg, 53%); yellow oil; R_f = 0.32 (petrol ether : EtOAc; 1 : 4); $[\alpha]_D^{25} = +7.30$ ($c = 1.0$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1743 (s, C=O), 1706 (s, C=O), 1651 (s, C=O), 1625 (s, C=C), 1581 (s, C=C), 1564 (s, C=C); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): a 0.28 : 1 mixture of AB/CD tautomers: δ (OH not

observed), 7.95 (d, $J = 16.0$ Hz, 1H, H1''), 7.74 (d, $J = 16.0$ Hz, 1H, H2''), 7.67 (dd, $J = 7.5$, 1.9 Hz, 2H, H4''), 7.56 (d, $J = 7.0$ Hz, 2H, H2'), 7.29 – 7.45 (m, 6H, H3', H4', H5'' and H6''), 6.49 (s, H2 minor tautomer), 6.45 (s, 1H, H2 major tautomer), 4.69 – 4.79 (m, 1H, H11), 3.97 (d, $J = 11.3$ Hz, 1H, H4_B), 3.12 (d, $J = 11.3$ Hz, 1H, H4_A), 1.55 – 1.65 (m, 4H, H12), 1.16 – 1.39 (m, 6H, H14 and H13); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): major CD tautomer: δ 187.9 (C6), 176.3 (C9), 175.9 (C8), 166.0 (C10), 146.7 (C2''), 138.5 (C1'), 134.3 (C3''), 131.8 (C4''), 129.4, 129.2 (C3' and C5''), 128.6, 128.5 (C4' and C6''), 127.2 (C2'), 117.4 (C1''), 99.6 (C7), 82.6 (C5), 75.8 (C11), 63.9 (C2), 35.4 (C4), 31.1 (C12), 31.0 (C12), 25.3 (C14), 23.6 (C13), 23.5 (C13); minor AB tautomer: δ 196.8 (C6), 176.5 (C9), (C8 not shown), 169.1 (C10), 147.3 (C2''), 139.1 (C1'), 134.3 (C3''), 132.0 (C4''), 129.5, 129.3 (C3' and C5''), 128.5, 128.3 (C4' and C6''), 127.2 (C2'), 116.7 (C1''), 101.9 (C7), 80.9 (C5), 75.9 (C11), 64.3 (C2), 35.5 (C4), 31.1 (C12), 31.0 (C12), 25.3 (C14), 23.6 (C13), 23.5 (C13); m/z (ESI⁻) 488 ([M-H]⁻, 100%); HRMS (ESI⁻) m/z calcd for $\text{C}_{28}\text{H}_{26}\text{O}_5\text{NS}$ [M-H]⁻ 488.1537; found 488.1542.

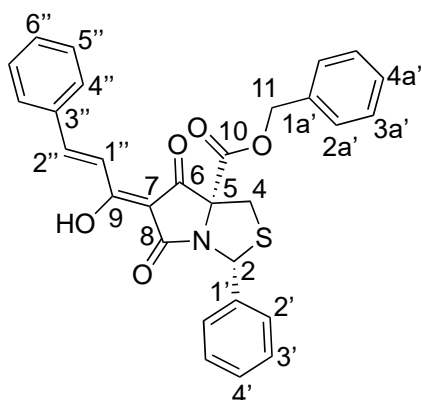
(2*R*,5*R*)-1-Aza-5-benzyloxycarbonyl-2-(5-bromopyridin-2-yl)-7-[(*Z*,2*E*)-1-hydroxy-3-phenylallylidene]-6,8-dioxo-3-thiabicyclo[3.3.0]-octane, 141c



Synthesised from **105c** (1 eqv. 38 mg, 0.07 mmol) with β -styrylmagnesium bromide; yield (12 mg, 29%); bright yellow oil; $R_f = 0.16$ (petrol: EtOAc; 2 : 3); $[\alpha]_D^{25} = +125.7$ ($c = 1.20$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1747 (s, C=O), 1706 (s, C=O), 1651 (s, C=O), 1625 (s, C=C), 1581 (s, C=C), 1563 (s, C=C); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): a 0.43 : 1

mixture of AB/CD tautomers: δ (OH not observed), 8.40 (d, $J = 2.1$ Hz, 1H, H6'), 7.99 (d, $J = 16.0$ Hz, 1H, H1''), 7.76 (d, $J = 16.0$ Hz, 1H, H2''), 7.72 – 7.65 (m, 3H, H4' and H4''), 7.53 – 7.29 (m, 5H, H3a', H5'' and H6''), 7.26 – 7.16 (m, 4H, H2a', H3' and H4a'), 6.44 (s, H2 minor), 6.40 (s, 1H, H2 major tautomer), 5.26 (d, $J = 12.2$ Hz, 1H, H11_B), 5.08 (d, $J = 12.2$ Hz, 1H, H11_A), 3.99 (d, $J = 11.5$ Hz, 1H, H4_B), 3.20 (d, $J = 11.5$ Hz, 1H, H4_A); $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 126 MHz): major CD tautomer: δ 187.4 (C6), 176.7 (C9), 176.5 (C8), 166.6 (C10), 156.5 (C2'), 150.4 (C6'), 147.4 (C2''), 139.4 (C4'), 134.7 (C1a'), 134.2 (C3''), 132.1 (C4''), 129.5, 129.3 (C5''), 128.8, 128.7, 128.7 (C2a', C3a', C4a' and C6''), 121.6 (C3'), 120.0 (C5'), 117.2 (C1''), 102.8 (C7), 83.3 (C5), 68.7 (C11), 64.5 (C2), 35.9 (C4); minor AB tautomer: δ 196.1 (C6), 177.0 (C9), 175.5 (C8), 166.9 (C10), 156.7 (C2'), 151.4 (C6'), 148.0 (C2''), 139.5 (C4'), 134.6 (C1a'), 134.4 (C3''), 131.7 (C4''), 129.6, 129.2 (C5''), 128.8, 128.7, 128.6 (C2a', C3a', C4a' and C6''), 122.8 (C3'), 120.2 (C5'), 117.7 (C1''), 99.3 (C7), 85.3 (C5), 68.8 (C11), 63.2 (C2), 33.7 (C4); m/z (ESI⁻) 575 and 577 ([M-H]⁻, 100%); HRMS (ESI⁻) m/z calcd for $\text{C}_{28}\text{H}_{20}\text{O}_5\text{N}_2\text{BrS}$ [M-H]⁻ 575.0282 and 577.0261; found 575.0275 and 577.0258.

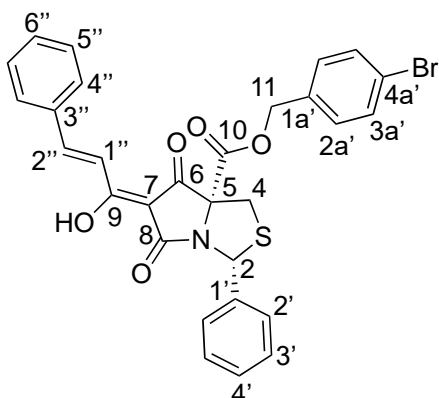
(2*R*,5*R*)-1-Aza-5-benzyloxycarbonyl-7-[(*Z*,2*E*)-1-hydroxy-3-phenylallylidene]-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]-octane, 141e



Synthesised from **105e** (1 eqv. 25 mg, 0.05 mmol) with β -styrylmagnesium bromide; yield (15 mg, 55%); bright yellow oil; $R_f = 0.27$ (petrol: EtOAc; 3 : 7); $[\alpha]_D^{25} = +56.3$ ($c = 0.90$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1747 (s, C=O), 1705 (s, C=O), 1652 (s, C=O), 1624 (s, C=C), 1580 (s, C=C), 1562 (s, C=C); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): a 0.26 : 1 mixture of

AB/CD tautomers: δ (OH not observed), 7.96 (d, $J = 15.9$ Hz, 1H, H1''), 7.73 (d, $J = 15.9$ Hz, 1H, H2''), 7.64 – 7.69 (m, 2H, H4''), 7.47 – 7.51 (m, 2H, H2'), 7.29 – 7.47 (m, 8H, H3', H3a', H4', H5'' and H6''), 7.12 – 7.20 (m, 3H, H2a' and H4a'), 6.49 (s, H2 minor tautomer), 6.44 (s, 1H, H2 major tautomer), 5.11 (d, $J = 12.3$ Hz, 1H, H11_B), 5.05 (d, $J = 12.3$ Hz, 1H, H11_A), 4.00 (d, $J = 11.4$ Hz, 1H, H4_B), 3.15 (d, $J = 11.4$ Hz, 1H, H4_A); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): major CD tautomer only: δ 187.4 (C6), 176.3 (C9), 176.2 (C8), 166.8 (C10), 146.9 (C2''), 138.3 (C1'), 134.7 (C1a'), 134.3 (C3''), 131.9 (C4''), 129.5, 129.3 (C3' and C5''), 128.7, 128.7, 128.6, 128.6, 128.5 (C2a', C3a', C4', C4a' and C6''), 127.2 (C2'), 117.4 (C1''), 99.6 (C7), 82.5 (C5), 68.7 (C11), 64.2 (C2), 35.6 (C4); m/z (ESI⁻) 496 ($[\text{M}-\text{H}]^-$, 100%); HRMS (ESI⁻) m/z calcd for $\text{C}_{29}\text{H}_{22}\text{O}_5\text{NS}$ $[\text{M}-\text{H}]^-$ 496.1224; found 496.1216.

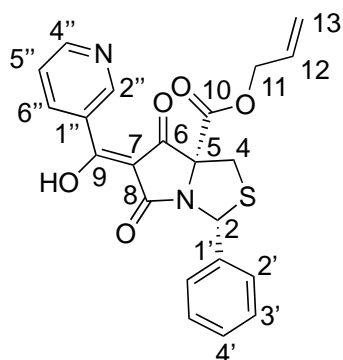
(2*R*,5*R*)-1-Aza-5-(4-bromobenzyloxycarbonyl)-7-[(*Z*,2*E*)-1-hydroxy-3-phenylallylidene]-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]-octane, 142e



Synthesised from **109e** (1 eqv. 40 mg, 0.07 mmol) with β -styrylmagnesium bromide; yield (25 mg, 59%); bright yellow oil; $R_f = 0.38$ (petrol: EtOAc; 3 : 7); $[\alpha]_D^{25} = +67.0$ ($c = 0.90$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1747 (s, C=O), 1706 (s, C=O), 1651 (s, C=O), 1625 (s, C=C), 1581 (s, C=C), 1563 (s, C=C); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): a 0.23 :

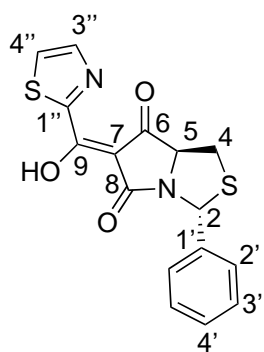
1 mixture of AB/CD tautomers: δ (OH not observed), 7.96 (d, $J = 16.0$ Hz, 1H, H1''), 7.71 (d, $J = 16.0$ Hz, 1H, H2''), 7.63 – 7.70 (m, 2H, H4''), 7.41 – 7.51 (m, 6H, H2', H3' and H5''), 7.40 (d, $J = 8.4$ Hz, 2H, H3a'), 7.25 – 7.30 (m, 2H, H4' and H6''), 6.99 (d, $J = 8.4$ Hz, 2H, H2a'), 6.49 (s, H2 minor tautomer), 6.44 (s, 1H, H2 major tautomer), 5.01 (s, 2H, H11), 3.98 (d, $J = 11.4$ Hz, 1H, H4_B), 3.14 (d, $J = 11.4$ Hz, 1H, H4_A); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): major CD tautomer only: δ 187.3 (C6), 176.3 (C9), 176.2 (C8), 166.7 (C10), 147.1 (C2''), 138.2 (C1'), 134.2 (C3''), 133.7 (C1a'), 132.0 (C3a'), 131.8 (C4''), 130.1 (C2a'), 129.5, 129.3 (C3' and C5''), 128.6, 128.6 (C4' and C6''), 127.2 (C2'), 122.7 (C4a'), 117.3 (C1''), 99.4 (C7), 82.3 (C5), 67.7 (C11), 64.0 (C2), 35.6 (C4); m/z (ESI⁻) 574 and 576 ([M-H]⁻, 100%); HRMS (ESI⁻) m/z calcd for $\text{C}_{29}\text{H}_{21}\text{O}_5\text{NBrS}$ [M-H]⁻ 574.0329 and 576.0311; found 574.0319 and 576.0295.

(2*R*,5*R*)-5-Allyloxycarbonyl-1-aza-7-[(*Z*)-hydroxy(pyridin-3-yl)methylene]-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]-octane, 143e



Synthesised from **102e** (1 eqv. 170 mg, 0.4 mmol) with pyridin-3-ylmagnesium bromide; yield (21 mg, 12%); orange-red oil; $R_f = 0.38$ (100% EtOAc); $[\alpha]_D^{25} = +73.1$ ($c = 1.04$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1741 (s, C=O), 1687 (s, C=O), 1631 (s, C=C); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ (*OH* not observed), 9.38 (s, 1H, H2''), 8.83 (s, 2H, H4'' and H6''), 7.80 – 7.73 (m, 1H, H5''), 7.56 (d, $J = 7.2$ Hz, 2H, H2'), 7.35 – 7.27 (m, 3H, H3' and H4'), 6.47 (s, 1H, H2), 5.83 – 5.69 (m, 1H, H12), 5.24 (dd, $J = 17.2$, 1.5 Hz, 1H, H13_B), 5.15 (dd, $J = 10.4$, 1.5 Hz, 1H, H13_A), 4.56 (d, $J = 5.9$ Hz, 2H, H11), 3.95 (d, $J = 11.3$ Hz, 1H, H4_B), 3.15 (d, $J = 11.3$ Hz, 1H, H4_A); $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 126 MHz): CD tautomer: δ 189.6 (C6), 181.6 (C9), 176.2 (C8), 168.4 (C10), 151.5 (C2''), 148.5 (C4''), 144.0 (C1''), 140.2 (C1'), 138.5 (C6''), 131.6 (C12), 128.4 (C3' and C4'), 127.0 (C2'), 124.5 (C5''), 118.9 (C13), 99.4 (C7), 81.3 (C5), 66.9 (C11), 64.8 (C2), 36.3 (C4); m/z (ESI⁺) 423 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for $\text{C}_{22}\text{H}_{19}\text{O}_5\text{NS}$ [M+H]⁺ 423.1009; found 423.1007.

(2*R*,5*S*)-1-aza-7-[(*Z*)-hydroxy(thiazol-2-yl)methylene]-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]-octane, 144e

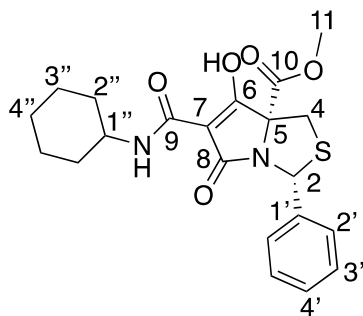


Synthesised from **102e** (1 eqv. 82 mg, 0.2 mmol) with isopropyl(thiazol-2-yl)magnesium; yield (16 mg, 23%); yellow oil; $R_f = 0.23$ (EtOAc : MeOH; 7 : 1); $[\alpha]_D^{25} = +5.63$ ($c = 0.84$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1703 (s, C=O), 1613 (s, C=C); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ (*OH* not observed), 8.02 (d, $J = 3.3$ Hz, 1H, H3''), 7.85 (d, $J = 3.3$ Hz, 1H, H4''), 7.49 (d, $J = 7.4$ Hz, 2H, H2'), 7.34 (t, $J = 7.4$ Hz, 2H, H3'), 7.28 (d, $J = 7.4$ Hz, 1H,

H4'), 6.47 (s, 1H, H2), 4.72 (app t, $J = 7.5$ Hz, 1H, H5), 3.36 (dd, $J = 11.1, 7.2$ Hz, 1H, H4_B), 3.08 (dd, $J = 11.1, 8.1$ Hz, 1H, H4_A); ¹³C NMR (CDCl₃, 101 MHz): CD tautomer: δ 189.2 (C6), 177.9 (C9), 173.0 (C8), 156.9 (C1''), 151.5 (C4''), 138.6 (C1'), 131.0 (C3''), 128.7 (C3'), 127.1 (C4'), 126.6 (C2'), 91.0 (C7), 67.6 (C5), 63.2 (C2), 33.9 (C4); m/z (ESI⁻) 343 ([M-H]⁻, 100%); HRMS (ESI⁺) m/z calcd for C₁₆H₁₂O₃N₂NaS₂ [M+Na]⁺ 367.0182; found 367.0187.

Tetramate carboxamides **145-159** were synthesised from the respective tetramate Weinreb amides **101-109** by general procedure **D** (section 6.2.4) with the corresponding amine (R²NH₂).

(2*R*,5*R*)-1-Aza-7-(cyclohexylaminocarbonyl)-6-hydroxy-5-methoxycarbonyl-8-oxo-2-phenyl-3-thiabicyclo[3.3.0]oct-6-ene, 145e

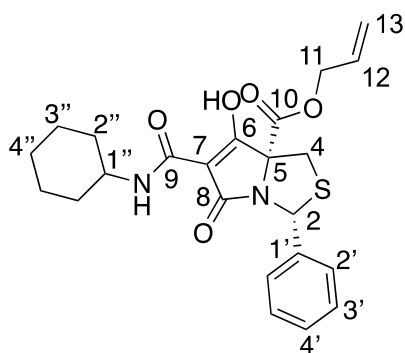


Synthesised from **101e** (1 eqv. 60 mg, 0.2 mmol) with cyclohexylamine; yield (20 mg, 30%); yellow oil; $R_f = 0.72$ (100% EtOAc); $[\alpha]_D^{25} = +147$ ($c = 1.00$, CHCl₃); $\nu_{\max}/\text{cm}^{-1}$ 1746 (s, C=O), 1691 (s, C=O), 1624 (s, C=C); ¹H NMR (CDCl₃, 400 MHz): a mixture of 1 : 1 AB/CD tautomers: δ (OH not

observed), 7.79 (br. s, 1H, NH), 7.72 (br. s, 1H, NH), 7.51 (d, $J = 7.3$ Hz, 4H, H2' AB and CD), 7.33 (t, $J = 7.3$ Hz, 4H, H3' AB and CD), 7.27 (d, $J = 7.3$ Hz, 2H, H4' AB and CD), 6.31 (s, 1H, H2 CD), 6.27 (s, 1H, H2 AB), 3.89 – 3.80 (m, 4H, H1'' and H4_B, AB and CD), 3.70 (s, 3H, H11 AB), 3.67 (s, 3H, H11 CD), 3.11 (d, $J = 11.5$ Hz, 2H, H4_A, AB and CD), 2.00 – 1.87 (m, 4H, H3''), 1.82 – 1.71 (m, 4H, H3''), 1.68 – 1.55 (m, 4H, H2''), 1.44 – 1.28 (m, 8H, H2'' and H4''); ¹³C{¹H} NMR (CDCl₃, 126 MHz): δ 189.0 (C6 CD), 187.1 (C6 AB), 178.4 (C8 CD), 173.2 (C8 AB), 168.0 (C10 CD), 167.7 (C10 AB), 165.9 (C9 CD), 165.8 (C9 AB), 139.2, 139.1 (C1'),

128.6, 128.6 (C3'), 128.3 (C4'), 127.0 (C2'), 89.4, 83.7 (C7), 82.6, 80.9 (C5), 63.9, 63.7 (C2), 53.6, 53.4 (C11), 50.0, 49.3 (C1''), 35.3, 35.2 (C4), 32.6, 32.6 (C2''), 25.2, 25.2 (C3''), 24.4 (C4''); m/z (ESI⁻) 415 ([M-H]⁻, 100%); HRMS (ESI⁻) m/z calcd for C₂₁H₂₃O₅N₂S [M-H]⁻ 415.1333; found 415.1340.

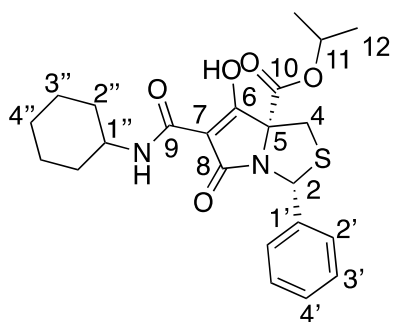
(2*R*,5*R*)-5-Allyloxycarbonyl-1-aza-7-(cyclohexylaminocarbonyl)-6-hydroxy-8-oxo-2-phenyl-3-thiabicyclo[3.3.0]oct-6-ene, 146e



Synthesised from **102e** (1 eqv. 80 mg, 0.2 mmol) with cyclohexylamine; yield (42 mg, 48%); yellow oil; R_f = 0.69 (100% EtOAc); $[\alpha]_D^{25}$ = +92.6 (c = 1.05, CHCl₃); $\nu_{\max}/\text{cm}^{-1}$ 1746 (s, C=O), 1691 (s, C=O), 1625 (s, C=C); ¹H NMR (CDCl₃, 400 MHz): a mixture of 0.9 : 1 AB/CD tautomers:

δ (OH not observed), 7.78 (br. s, 1H, NH), 7.71 (br. s, 1H, NH), 7.52 (d, J = 7.4 Hz, 4H, H2' AB and CD), 7.34 – 7.23 (m, 6H, H3' and H4' AB and CD), 6.31 (s, 1H, H2 CD), 6.26 (s, 1H, H2 AB), 5.82 – 5.68 (m, 2H, H12 AB and CD), 5.30 – 5.15 (m, 4H, H13 AB and CD), 4.64 – 4.49 (m, 4H, H11 AB and CD), 3.92 – 3.78 (m, 4H, H1'' and H4_B, AB and CD), 3.11 (d, J = 11.5 Hz, 2H, H4_A AB and CD), 1.98 – 1.90 (m, 4H, H3''), 1.80 – 1.72 (m, 4H, H3''), 1.65 – 1.57 (m, 4H, H2''), 1.41 – 1.28 (m, 8H, H2'' and H4''); ¹³C NMR (CDCl₃, 101 MHz): δ 188.8 (C6 CD), 187.0 (C6 AB), 178.5 (C8 CD), 173.3 (C8 AB), 167.1 (C10 CD), 166.8 (C10 AB), 165.9 (C9 CD), 165.7 (C9 AB), 139.1, 139.1 (C1'), 131.2, 131.1 (C12), 128.5 (C3'), 128.2 (C4'), 127.0 (C2'), 119.4, 119.2 (C13), 89.5, 83.7 (C7), 82.7, 81.0 (C5), 67.3, 67.1 (C11), 63.9, 63.7 (C2), 49.9, 49.2 (C1''), 35.3, 35.2 (C4), 32.6 (C2''), 25.2, 25.1 (C3''), 24.4 (C4''); m/z (ESI⁻) 441 ([M-H]⁻, 100%); HRMS (ESI⁻) m/z calcd for C₂₃H₂₅O₅N₂S [M-H]⁻ 441.1490; found 441.1494.

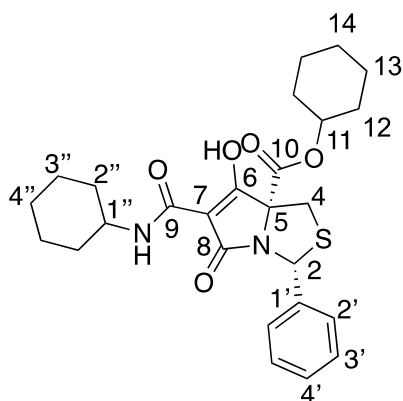
(2*R*,5*R*)-1-Aza-7-(cyclohexylaminocarbonyl)-6-hydroxy-5-isopropoxy-carbonyl-8-oxo-2-phenyl-3-thiabicyclo[3.3.0]oct-6-ene, 147e



Synthesised from **103e** (1 eqv. 34 mg, 0.08 mmol) with cyclohexylamine; yield (18 mg, 49%); yellow oil; $R_f = 0.47$ (100% EtOAc); $[\alpha]_D^{25} = +141$ ($c = 0.90$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1739 (s, C=O), 1693 (s, C=O), 1627 (s, C=C); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): a mixture of 1.1 : 1 AB/CD tautomers: δ

7.78 (br. s, 1H, NH), 7.68 (br. s, 1H, NH), 7.54 (d, $J = 7.2$ Hz, 4H, H2' AB and CD), 7.35 – 7.27 (m, 6H, H3' and H4' AB and CD), 6.31 (s, 1H, H2 CD), 6.26 (s, 1H, H2 AB), 5.68 (br. s, 2H, OH AB and CD), 5.05 – 4.93 (m, 2H, H11 AB and CD), 3.92 – 3.80 (m, 4H, H1'' and H4_B, AB and CD), 3.09 (app dd, $J = 11.5, 1.3$ Hz, 2H, H4_A AB and CD), 1.99 – 1.93 (m, 4H, H3''), 1.81 – 1.74 (m, 4H, H3''), 1.69 – 1.58 (m, 4H, H2''), 1.42 – 1.31 (m, 8H, H2'' and H4''), 1.17 (app t, $J = 6.2$ Hz, 6H, H12_B AB and CD), 1.11 (d, $J = 6.2$ Hz, 3H, H12_A), 1.08 (d, $J = 6.2$ Hz, 3H, H12_A); $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 126 MHz): δ 188.6 (C6 CD), 187.4 (C6 AB), 178.7 (C8 CD), 173.4 (C8 AB), 166.9 (C10 CD), 166.5 (C10 AB), 165.9 (C9 CD), 165.7 (C9 AB), 139.3, 139.3 (C1'), 128.5, 128.5 (C3'), 128.2, 128.2 (C4'), 127.1, 127.0 (C2'), 90.2, 83.7 (C7), 82.9, 81.0 (C5), 71.1, 70.7 (C11), 63.8, 63.6 (C2), 50.0, 49.1 (C1''), 35.3 (C4), 32.7, 32.6 (C2''), 25.3, 25.2 (C3''), 24.5 (C4''), 21.6, 21.6 (C12_B), 21.5, 21.4 (C12_A); m/z (ESI⁺) 445 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for $\text{C}_{23}\text{H}_{29}\text{O}_5\text{N}_2\text{S}$ [M+H]⁺ 445.1792; found 445.1791.

(2*R*,5*R*)-1-Aza-5-cyclohexyloxycarbonyl-7-(cyclohexylaminocarbonyl)-6-hydroxy-8-oxo-2-phenyl-3-thiabicyclo[3.3.0]oct-6-ene, 148e

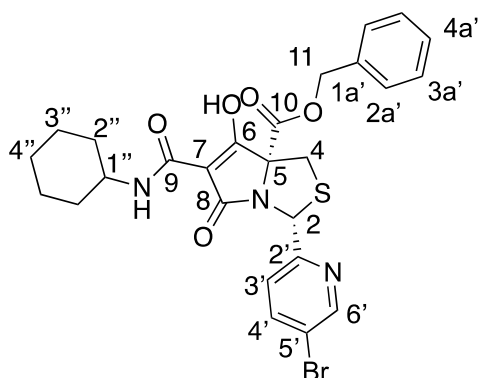


Synthesised from **104e** (1 eqv. 48 mg, 0.1 mmol) with cyclohexylamine; yield (32 mg, 61%); yellow oil; $R_f = 0.70$ (100% EtOAc); $[\alpha]_D^{25} = +185$ ($c = 1.20$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1739 (s, C=O), 1692 (s, C=O), 1626 (s, C=C); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): a mixture of 1.1 : 1 AB/CD tautomers: δ (OH not observed), 7.78 (br. s, 1H, NH), 7.67 (br. s, 1H,

NH), 7.54 (d, $J = 8.0$ Hz, 4H, H2' AB and CD), 7.35 – 7.25 (m, 6H, H3' and H4' AB and CD), 6.30 (s, 1H, H2 CD), 6.25 (s, 1H, H2 AB), 4.85 – 4.71 (m, 2H, H11 AB and CD), 3.91 – 3.79 (m, 4H, H1'' and H4_B, AB and CD), 3.09 (d, $J = 11.5$ Hz, 2H, H4_A AB and CD), 2.00 – 1.89 (m, 4H), 1.83 – 1.71 (m, 8H), 1.71 – 1.54 (m, 8H), 1.54 – 1.27 (m, 20H) (H2'', 3'', 4'', H12, 13, 14, AB and CD, a total of 40 H); $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 126 MHz): δ 188.6 (C6 CD), 187.5 (C6 AB), 178.7 (C8 CD), 173.4 (C8 AB), 166.8 (C10 CD), 166.4 (C10 AB), 165.9 (C9 CD), 165.7 (C9 AB), 139.3 (C1'), 128.5, 128.5 (C3'), 128.2, 128.2 (C4'), 127.0 (C2'), 90.4, 83.8 (C7), 83.1, 81.1 (C5), 75.8, 75.4 (C11), 63.9, 63.7 (C2), 50.0, 49.1 (C1''), 35.3, 35.2 (C4), 32.7, 32.6 (C2''), 31.2, 31.2, 31.2, 31.2 (C12), 25.4, 25.3, 25.3, 25.2 (C3'', C14), 24.5 (C4''), 23.6, 23.6 (C13); m/z (ESI⁻) 483 ([M-H]⁻, 100%); HRMS (ESI⁻) m/z calcd for $\text{C}_{26}\text{H}_{31}\text{O}_5\text{N}_2\text{S}$ [M-H]⁻ 483.1948; found 483.1969.

(2*R*,5*R*)-1-Aza-5-benzyloxycarbonyl-2-(5-bromopyridin-2-yl)-7-

(cyclohexylaminocarbonyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 149c



Synthesised from **105c** (1 eqv. 83 mg, 0.2 mmol)

with cyclohexylamine; yield (31 mg, 35%); yellow

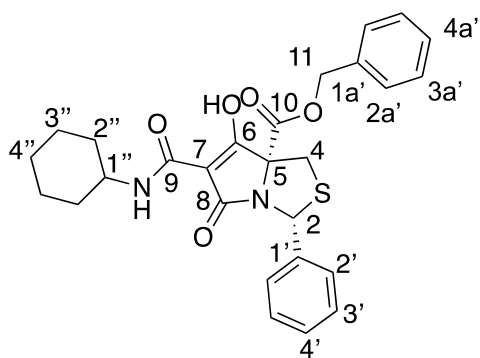
oil; $R_f = 0.71$ (100% EtOAc); $[\alpha]_D^{25} = +96.8$ ($c = 1.02$,

CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1743 (s, C=O), 1692 (s, C=O),

1625 (s, C=C); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): a mixture

of 0.9 : 1 AB/CD tautomers: δ (OH not observed), 8.59 – 8.53 (m, 1H, H6'), 8.35 (d, $J = 2.0$ Hz, 1H, H6'), 7.87 (br. s, 1H, NH), 7.78 (dd, $J = 8.2, 2.4$ Hz, 2H, H4' AB and CD), 7.69 (br. s, 1H, NH), 7.49 – 7.44 (m, 2H, H3' AB and CD), 7.38 – 7.29 (m, 8H, H2a' and H3a', AB and CD), 7.25 – 7.22 (m, 2H, H4a' AB and CD), 6.27 (s, 1H, H2 CD), 6.24 (s, 1H, H2 AB), 5.27 (d, $J = 12.3$ Hz, 2H, H11_B AB and CD), 5.08 (app dd, $J = 12.3, 6.6$ Hz, 2H, H11_A AB and CD), 3.91 (app dd, $J = 11.6, 4.0$ Hz, 2H, H4_B AB and CD), 3.88 – 3.79 (m, 2H, H1'' AB and CD), 3.17 (d, $J = 11.6$ Hz, 2H, H4_A AB and CD), 1.99 – 1.93 (m, 4H, H3''), 1.81 – 1.72 (m, 4H, H3''), 1.67 – 1.59 (m, 4H, H2''), 1.44 – 1.35 (m, 8H, H2'' and H4''); $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 126 MHz): δ 189.4 (C6 CD), 187.1 (C6 AB), 178.6 (C8 CD), 173.5 (C8 AB), 167.5 (C10 CD), 167.3 (C10 AB), 165.8 (C9 CD), 165.8 (C9 AB), 157.4, 157.3 (C2'), 151.2, 150.1 (C6'), 139.3 (C4'), 135.0, 134.8 (C1a'), 128.7, 128.7 (C3a', C4a' and C2a'), 122.8, 121.5 (C3'), 120.0, 119.7 (C5'), 88.8, 85.2 (C7), 83.6, 81.9 (C5), 68.5, 68.3 (C11), 64.1, 63.0 (C2), 50.0, 49.4 (C1''), 35.6, 35.5 (C4), 32.6, 32.6 (C2''), 25.2, 25.1 (C3''), 24.4 (C4''); m/z (ESI⁻) 570 and 572 ([M-H]⁻, 100%); HRMS (ESI⁻) m/z calcd for $\text{C}_{26}\text{H}_{25}\text{O}_5\text{N}_3\text{BrS}$ [M-H]⁻ 570.0704 and 572.0685; found 570.0700 and 572.0678.

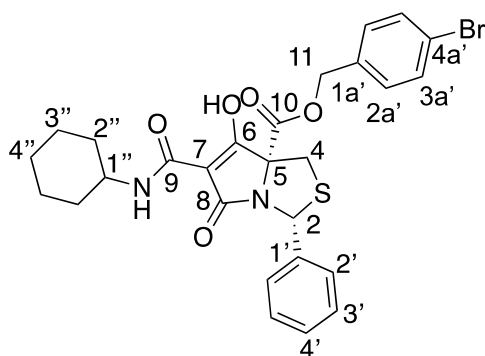
(2*R*,5*R*)-1-Aza-5-benzyloxycarbonyl-7-(cyclohexylaminocarbonyl)-6-hydroxy-8-oxo-2-phenyl-3-thiabicyclo[3.3.0]oct-6-ene, 149e



Synthesised from **105e** (1 eqv. 37 mg, 0.08 mmol) with cyclohexylamine; yield (14 mg, 35%); pale yellow oil; $R_f = 0.68$ (100% EtOAc); $[\alpha]_D^{25} = +62.9$ ($c = 0.70$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1744 (s, C=O), 1693 (s, C=O), 1625 (s, C=C); $^1\text{H NMR}$ (CDCl_3 , 500 MHz): a

mixture of 0.9 : 1 AB/CD tautomers: δ 7.78 (br. s, 1H, NH), 7.69 (br. s, 1H, NH), 7.51 – 7.41 (m, 4H, H2' AB and CD), 7.36 – 7.29 (m, 6H, H3' and H4' AB and CD), 7.25 – 7.16 (m, 10H, H2a', H3a' and H4a', AB and CD), 6.49 (br. s, 2H, OH AB and CD), 6.29 (s, 1H, H2 CD), 6.25 (s, 1H, H2 AB), 5.18 – 5.06 (m, 4H, H11 AB and CD), 3.90 (app dd, $J = 11.5, 8.5$ Hz, 2H, H4_B AB and CD), 3.78 – 3.71 (m, 2H, H1'' AB and CD), 3.12 (d, $J = 11.5$ Hz, 2H, H4_A AB and CD), 2.01 – 1.88 (m, 4H), 1.88 – 1.81 (m, 2H), 1.81 – 1.72 (m, 4H), 1.67 – 1.59 (m, 2H), 1.41 – 1.33 (m, 8H), (H2'', 3'', 4'', AB and CD, a total of 20 H); $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 126 MHz): δ 188.9 (C6 CD), 187.1 (C6 AB), 178.7 (C8 CD), 173.5 (C8 AB), 167.5 (C10 CD), 167.2 (C10 AB), 165.9 (C9 CD), 165.8 (C9 AB), 139.1, 139.0 (C1'), 135.0, 134.8 (C1a'), 128.7, 128.6, 128.6, 128.6, 128.5, 128.5, 128.5, 128.5, 128.4, 128.2 (C3', C4', C3a', C4a' and C2a'), 127.0 (C2'), 89.6, 83.8 (C7), 82.9, 81.2 (C5), 68.6, 68.3 (C11), 64.2, 63.9 (C2), 50.0, 49.3 (C1''), 35.4, 35.3 (C4), 32.7, 32.6 (C2''), 25.2, 25.2 (C3''), 24.4 (C4''); m/z (ESI⁺) 493 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for $\text{C}_{27}\text{H}_{29}\text{O}_5\text{N}_2\text{S}$ [M+H]⁺ 493.1792; found 493.1791.

(2*R*,5*R*)-1-Aza-5-(4-bromobenzoyloxycarbonyl)-7-(cyclohexylaminocarbonyl)-6-hydroxy-8-oxo-2-phenyl-3-thiabicyclo[3.3.0]oct-6-ene, 150e



Synthesised from **109e** (1 eqv. 24 mg, 0.04 mmol)

with cyclohexylamine; yield (12 mg, 47%); pale

yellow oil; $R_f = 0.60$ (100% EtOAc); $[\alpha]_D^{25} = +148$ (c

$= 0.60$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1744 (s, C=O), 1692 (s,

C=O), 1625 (s, C=C); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): a

mixture of 0.9 : 1 AB/CD tautomers: δ 7.77 (br. s, 1H, NH), 7.71 (br. s, 1H, NH), 7.49 – 7.37

(m, 8H, H2' and H3a', AB and CD), 7.26 – 7.19 (m, 6H, H3' and H4', AB and CD), 7.09 – 6.98

(m, 4H, H2a' AB and CD), 6.29 (s, 1H, H2 CD), 6.25 (s, 1H, H2 AB), 5.13 – 4.97 (m, 4H, H11

AB and CD), 3.98 (br. s, 2H, OH AB and CD), 3.90 – 3.82 (m, 4H, H1'' and H4_B, AB and CD),

3.12 (d, $J = 11.5$ Hz, 2H, H4_A AB and CD), 2.00 – 1.89 (m, 4H, H3''), 1.83 – 1.70 (m, 4H,

H3''), 1.70 – 1.54 (m, 4H, H2''), 1.43 – 1.31 (m, 8H, H2'' and H4''); $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 126

MHz): δ 189.0 (C6 CD), 186.9 (C6 AB), 178.6 (C8 CD), 173.4 (C8 AB), 167.4 (C10 CD), 167.1

(C10 AB), 165.9 (C9 CD), 165.8 (C9 AB), 139.1, 139.0 (C1'), 134.0, 133.8 (C1a'), 131.8,

131.8 (C3a'), 130.1, 130.1 (C2a'), 128.5, 128.5 (C3'), 128.3, 128.3 (C4'), 127.0, 127.0 (C2'),

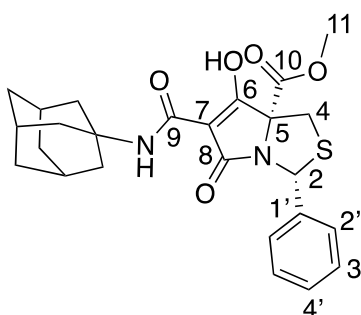
122.7, 122.6 (C4a'), 89.2, 83.7 (C7), 82.8, 81.1 (C5), 67.7, 67.4 (C11), 64.0, 63.8 (C2), 50.0,

49.3 (C1''), 35.4, 35.3 (C4), 32.6, 32.6 (C2''), 25.2, 25.2 (C3''), 24.4 (C4''); m/z (ESI⁻) 569

and 571 ([M-H]⁻, 100%); HRMS (ESI⁻) m/z calcd for $\text{C}_{27}\text{H}_{26}\text{O}_5\text{N}_2\text{BrS}$ [M-H]⁻ 569.0751 and

571.0732; found 569.0757 and 571.0732.

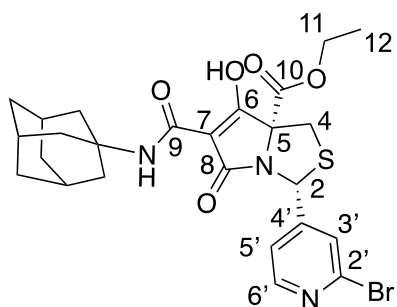
(2*R*,5*R*)-7-(Adamantylaminocarbonyl)-1-aza-6-hydroxy-5-methoxycarbonyl-8-oxo-2-phenyl-3-thiabicyclo[3.3.0]oct-6-ene, 151e



Synthesised from **101e** (1 eqv. 55 mg, 0.1 mmol) with 1-adamantylamine; yield (38 mg, 56%); greenish yellow oil; $R_f = 0.65$ (EtOAc : petrol; 4 : 1); $[\alpha]_D^{25} = +162$ ($c = 1.00$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1745 (s, C=O), 1688 (s, C=O), 1626 (s, C=C); ^1H NMR (CDCl_3 , 400 MHz): a mixture of 0.9 : 1 AB/CD

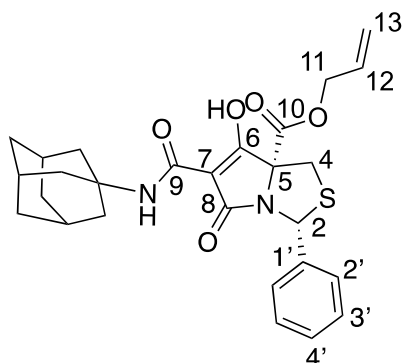
tautomers: δ (OH not observed), 7.82 (br. s, 1H, NH), 7.72 (br. s, 1H, NH), 7.51 (d, $J = 7.7$ Hz, 4H, H2' AB and CD), 7.37 – 7.27 (m, 6H, H3' and H4', AB and CD), 6.31 (s, 1H, H2 CD), 6.26 (s, 1H, H2 AB), 3.86 (d, $J = 11.5$ Hz, 2H, H4_B AB and CD), 3.70 (s, 3H, H11 AB), 3.67 (s, 3H, H11 CD), 3.10 (d, $J = 11.5$ Hz, 2H, H4_A AB and CD), 2.14 (br. s, 6H, Adamantyl-CH AB and CD), 2.05 (br. s, 12H, Adamantyl-CH₂ AB and CD), 1.69 (br. s, 12H, Adamantyl-CH₂ AB and CD); $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 126 MHz): δ 189.4 (C6 CD), 187.0 (C6 AB), 178.6 (C8 CD), 173.4 (C8 AB), 168.0 (C10 CD), 167.7 (C10 AB), 166.8 (C9 CD), 166.7 (C9 AB), 139.2, 139.1 (C1'), 128.6, 128.5 (C3'), 128.3 (C4'), 126.9 (C2'), 89.2, 84.0 (C7), 82.6, 81.0 (C5), 63.9, 63.8 (C2), 55.0, 54.5 (C11), 53.6, 53.4 (Adamantyl-C), 41.6 (Adamantyl-CH₂), 36.0, 36.0 (Adamantyl-CH₂), 35.3, 35.2 (C4), 29.4 (Adamantyl-CH); m/z (ESI⁻) 467 ([M-H]⁻, 100%); HRMS (ESI⁻) m/z calcd for $\text{C}_{25}\text{H}_{27}\text{O}_5\text{N}_2\text{S}$ [M-H]⁻ 467.1646; found 467.1653.

(2*R*,5*R*)-7-(Adamantylaminocarbonyl)-1-aza-2-(2-bromopyridin-4-yl)-5-ethoxycarbonyl-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 152b



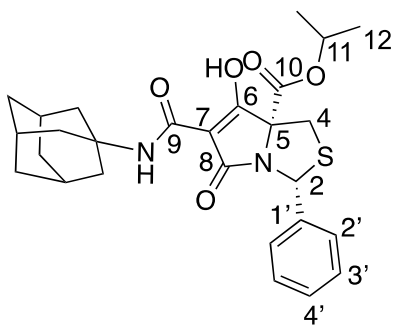
Synthesised from **107b** (1 eqv. 63 mg, 0.1 mmol) with 1-adamantylamine; yield (39 mg, 52%); yellow oil; $R_f = 0.30$ (100% EtOAc); $[\alpha]_D^{25} = +58.8$ ($c = 0.95$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1743 (s, C=O), 1689 (s, C=O), 1626 (s, C=C); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): a mixture of 1 : 1 AB/CD tautomers: δ 8.31 (d, $J = 5.1$ Hz, 2H, H6' AB and CD), 7.88 (br. s, 1H, NH), 7.68 (s, 2H, H3' AB and CD), 7.63 (br. s, 1H, NH), 7.38 (d, $J = 5.1$ Hz, 2H, H5' AB and CD), 6.18 (s, 1H, H2 CD), 6.14 (s, 1H, H2 AB), 5.61 (br. s, 2H, OH AB and CD), 4.37 – 4.10 (m, 4H, H11 AB and CD), 3.92 – 3.83 (m, 2H, H4_B AB and CD), 3.11 (app dd, $J = 11.5, 3.3$ Hz, 2H, H4_A AB and CD), 2.15 (br. s, 6H, Adamantyl-CH AB and CD), 2.06 (br. s, 12H, Adamantyl-CH₂ AB and CD), 1.71 (br. s, 12H, Adamantyl-CH₂ AB and CD), 1.24 – 1.18 (m, 6H, H12 AB and CD); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ 189.7 (C6 CD), 186.6 (C6 AB), 178.5 (C8 CD), 173.5 (C8 AB), 167.0 (C10), 166.8 (C9 CD), 166.7 (C9 AB), 151.5 (C6'), 150.3 (C4'), 142.7 (C2'), 127.1, 125.9 (C3'), 121.0, 120.9 (C5'), 87.1, 83.8 (C7), 82.7, 81.3 (C5), 63.4, 63.2 (C2), 61.7, 61.6 (C11), 55.3, 54.8 (Adamantyl-C), 41.7 (Adamantyl-CH₂), 36.0, 35.9 (Adamantyl-CH₂), 35.8, 35.7 (C4), 29.4 (Adamantyl-CH), 14.1 (C12); m/z (ESI⁻) 560 and 562 ($[\text{M}-\text{H}]^-$, 100%); HRMS (ESI⁻) m/z calcd for $\text{C}_{25}\text{H}_{27}\text{O}_5\text{N}_3\text{BrS}$ $[\text{M}-\text{H}]^-$ 560.0849 and 562.0830; found 560.0852 and 562.0828.

(2*R*,5*R*)-7-(Adamantylaminocarbonyl)-5-allyloxycarbonyl-1-aza-6-hydroxy-8-oxo-2-phenyl-3-thiabicyclo[3.3.0]oct-6-ene, 153e



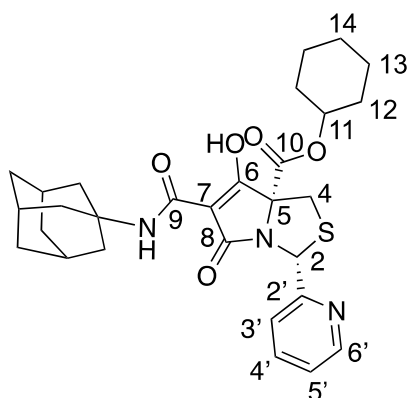
Synthesised from **102e** (1 eqv. 76 mg, 0.2 mmol) with 1-adamantylamine; yield (43 mg, 46%); pale yellow oil; $R_f = 0.76$ (EtOAc : petrol; 7 : 3); $[\alpha]_D^{25} = +53.9$ ($c = 1.08$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1745 (s, C=O), 1689 (s, C=O), 1626 (s, C=C); ^1H NMR (CDCl_3 , 400 MHz): a mixture of 1 : 1 AB/CD tautomers: δ (OH not observed), 7.81 (br. s, 1H, NH), 7.71 (br. s, 1H, NH), 7.52 (d, $J = 7.4$ Hz, 4H, H2' AB and CD), 7.33 – 7.26 (m, 6H, H3' and H4', AB and CD), 6.31 (s, 1H, H2 CD), 6.26 (s, 1H, H2 AB), 5.83 – 5.68 (m, 2H, H12 AB and CD), 5.30 – 5.16 (m, 4H, H13 AB and CD), 4.64 – 4.49 (m, 4H, H11 AB and CD), 3.87 (app dd, $J = 11.5, 6.5$ Hz, 2H, H4_B AB and CD), 3.10 (d, $J = 11.5$ Hz, 2H, H4_A AB and CD), 2.14 (br. s, 6H, Adamantyl-CH AB and CD), 2.05 (br. s, 12H, Adamantyl-CH₂ AB and CD), 1.70 (br. s, 12H, Adamantyl-CH₂ AB and CD); ^{13}C NMR (CDCl_3 , 101 MHz): δ 189.3 (C6 CD), 187.0 (C6 AB), 178.8 (C8 CD), 173.5 (C8 AB), 167.2 (C10 CD), 166.9 (C10 AB), 166.8 (C9 CD), 166.7 (C9 AB), 139.2, 139.1 (C1'), 131.3, 131.1 (C12), 128.5, 128.5 (C3'), 128.2 (C4'), 127.0 (C2'), 119.4, 119.3 (C13), 89.4, 84.0 (C7), 82.7, 81.1 (C5), 67.4, 67.2 (C11), 63.9, 63.8 (C2), 55.0, 54.4 (Adamantyl-C), 41.7 (Adamantyl-CH₂), 36.0, 36.0 (Adamantyl-CH₂), 35.3, 35.3 (C4), 29.4 (Adamantyl-CH); m/z (ESI⁻) 493 ([M-H]⁻, 100%); HRMS (ESI⁻) m/z calcd for $\text{C}_{27}\text{H}_{29}\text{O}_5\text{N}_2\text{S}$ [M-H]⁻ 493.1803; found 493.1808.

(2*R*,5*R*)-7-(Adamantylaminocarbonyl)-1-aza-6-hydroxy-5-isopropoxy-8-oxo-2-phenyl-3-thiabicyclo[3.3.0]oct-6-ene, 154e



Synthesised from **103e** (1 eqv. 125 mg, 0.3 mmol) with 1-adamantylamine; yield (67 mg, 44%); pale yellow oil; $R_f = 0.76$ (EtOAc : petrol; 7 : 3); $[\alpha]_D^{25} = +133$ ($c = 0.96$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1739 (s, C=O), 1692 (s, C=O), 1629 (s, C=C); ^1H NMR (CDCl_3 , 400 MHz): a mixture of 1 : 1 AB/CD tautomers: δ (OH not observed), 7.80 (br. s, 1H, NH), 7.67 (br. s, 1H, NH), 7.53 (d, $J = 7.4$ Hz, 4H, H2' AB and CD), 7.34 – 7.23 (m, 6H, H3' and H4', AB and CD), 6.30 (s, 1H, H2 CD), 6.24 (s, 1H, H2 AB), 5.05 – 4.90 (m, 2H, H11 AB and CD), 3.85 (app dd, $J = 11.5, 8.3$ Hz, 2H, H4_B AB and CD), 3.07 (d, $J = 11.5$ Hz, 2H, H4_A AB and CD), 2.13 (br. s, 6H, Adamantyl-CH AB and CD), 2.05 (br. s, 12H, Adamantyl-CH₂ AB and CD), 1.69 (br. s, 12H, Adamantyl-CH₂ AB and CD), 1.16 (app t, $J = 6.3$ Hz, 6H, H12_B AB and CD), 1.08 (app dd, $J = 10.3, 6.3$ Hz, 6H, H12_A AB and CD); ^{13}C NMR (CDCl_3 , 101 MHz): δ 189.0 (C6 CD), 187.2 (C6 AB), 178.9 (C8 CD), 173.5 (C8 AB), 166.8 (C10 CD), 166.8 (C10 AB), 166.6 (C9 CD), 166.5 (C9 AB), 139.3, 139.3 (C1'), 128.4, 128.4 (C3'), 128.1 (C4'), 127.0, 127.0 (C2'), 89.9, 83.9 (C7), 82.9, 81.0 (C5), 71.0, 70.7 (C11), 63.7, 63.5 (C2), 54.9, 54.2 (Adamantyl-C), 41.6 (Adamantyl-CH₂), 36.0, 35.9 (Adamantyl-CH₂), 35.3 (C4), 29.4 (Adamantyl-CH), 21.6, 21.4 (C12); m/z (ESI⁻) 495 ([M-H]⁻, 100%); HRMS (ESI⁻) m/z calcd for $\text{C}_{27}\text{H}_{31}\text{O}_5\text{N}_2\text{S}$ [M-H]⁻ 495.1948; found 495.1964.

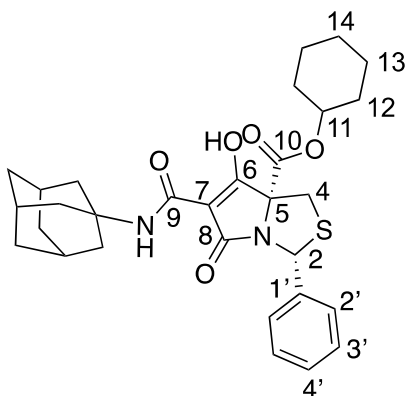
(2*R*,5*R*)-7-(Adamantylaminocarbonyl)-5-cyclohexyloxycarbonyl-1-aza-6-hydroxy-8-oxo-2-(pyridin-2-yl)-3-thiabicyclo[3.3.0]oct-6-ene, 155a



Synthesised from **104a** (1 eqv. 45 mg, 0.1 mmol) with 1-adamantylamine; yield (18 mg, 33%); yellow oil; $R_f = 0.65$ (EtOAc : petrol; 9 : 1); $[\alpha]_D^{25} = +64.3$ ($c = 0.40$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1730 (s, C=O), 1690 (s, C=O), 1631 (s, C=C); $^1\text{H NMR}$ (CDCl_3 , 600 MHz): a mixture of 1 : 1 AB/CD tautomers: δ (OH not observed), 8.56 – 8.49 (m,

2H, H6' AB and CD), 7.86 (s br. s, 1H, NH), 7.69 (br. s, 1H, NH), 7.67 (app d, $J = 5.8$ Hz, 4H, H4' and H3', AB and CD), 7.18 (t, $J = 5.8$ Hz, 2H, H5' AB and CD), 6.35 (s, 1H, H2 CD), 6.31 (s, 1H, H2 AB), 4.85 – 4.73 (m, 2H, H11 AB and CD), 3.87 (app dd, $J = 11.4, 7.5$ Hz, 2H, H4_B AB and CD), 3.12 (app dd, $J = 11.4, 2.8$ Hz, 2H, H4_A AB and CD), 2.15 (br. s, 6H, Adamantyl-CH AB and CD), 2.07 (br. s, 12H, Adamantyl-CH₂ AB and CD), 1.71 (br. s, 20H, Adamantyl-CH₂ and H12, AB and CD), 1.36 – 1.27 (m, 12H, H14 and H13, AB and CD); $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 151 MHz): δ 189.5 (C6 CD), 187.4 (C6 AB), 179.0 (C8 CD), 173.6 (C8 AB), 166.9 (C10 CD), 166.7 (C10 AB), 166.7 (C9 CD), 166.4 (C9 AB), 159.1 (C2'), 149.2 (C6'), 136.9 (C4'), 122.8 (C3'), 120.2 (C5'), 89.7, 84.0 (C7), 83.8, 81.9, (C5), 75.7, 75.4 (C11), 64.6, 64.3 (C2), 55.0, 54.4 (Adamantyl-C), 41.7, 41.7 (Adamantyl-CH₂), 36.1, 36.0 (Adamantyl-CH₂), 35.5, 35.5 (C4), 31.3, 31.3 (C12), 29.5 (Adamantyl-CH), 25.4, 25.4 (C14), 23.6 (C13); m/z (ESI⁻) 536 ([M-H]⁻, 100%); HRMS (ESI⁻) m/z calcd for $\text{C}_{29}\text{H}_{34}\text{O}_5\text{N}_3\text{S}$ [M-H]⁻ 536.2225; found 536.2220.

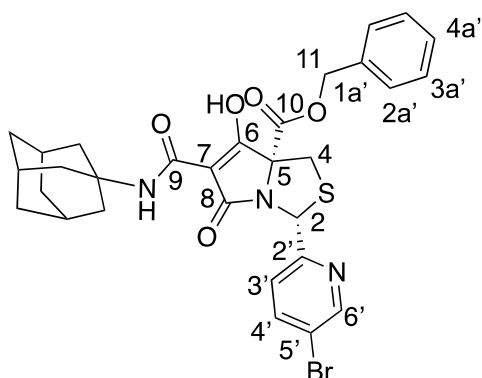
(2*R*,5*R*)-7-(Adamantylaminocarbonyl)-5-cyclohexyloxycarbonyl-1-aza-6-hydroxy-8-oxo-2-phenyl-3-thiabicyclo[3.3.0]oct-6-ene, 155e



Synthesised from **104e** (1 eqv. 115 mg, 0.3 mmol) with 1-adamantylamine; yield (78 mg, 57%); yellow oil; $R_f = 0.89$ (EtOAc : petrol; 7 : 3); $[\alpha]_D^{25} = +99.4$ ($c = 0.98$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1739 (s, C=O), 1690 (s, C=O), 1628 (s, C=C); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): a mixture of 1 : 1 AB/CD tautomers: δ 8.75 (br. s, 2H, OH AB and CD), 7.82

(br. s, 1H, NH), 7.68 (br. s, 1H, NH), 7.55 (d, $J = 7.6$ Hz, 4H, H2' AB and CD), 7.34 – 7.27 (m, 6H, H3' and H4', AB and CD), 6.30 (s, 1H, H2 CD), 6.25 (s, 1H, H2 AB), 4.84 – 4.71 (m, 2H, H11 AB and CD), 3.87 (app dd, $J = 11.4, 8.0$ Hz, 2H, H4_B AB and CD), 3.09 (d, $J = 11.4$ Hz, 2H, H4_A AB and CD), 2.14 (br. s, 6H, Adamantyl-CH AB and CD), 2.07 (br. s, 12H, Adamantyl-CH₂ AB and CD), 1.71 (br. s, 20H, Adamantyl-CH₂ and H12, AB and CD), 1.54 – 1.23 (m, 12H, H14 and H13, AB and CD); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ 189.1 (C6 CD), 187.3 (C6 AB), 178.9 (C8 CD), 173.5 (C8 AB), 166.8 (C10 CD), 166.7 (C10 AB), 166.5 (C9 CD), 166.4 (C9 AB), 139.3, 139.2 (C1'), 128.4, 128.4 (C3'), 128.1 (C4'), 126.9 (C2'), 90.0, 84.0 (C7), 83.0, 81.1 (C5), 75.7, 75.4 (C11), 63.8, 63.6 (C2), 54.8, 54.2 (Adamantyl-C), 41.6 (Adamantyl-CH₂), 36.0, 35.9 (Adamantyl-CH₂), 35.2 (C4), 31.2, 31.2, 31.1, 31.1 (C12), 29.4 (Adamantyl-CH), 25.3, 25.3 (C14), 23.6 (C13); m/z (ESI⁻) 535 ([M-H]⁻, 100%); HRMS (ESI⁻) m/z calcd for $\text{C}_{30}\text{H}_{35}\text{O}_5\text{N}_2\text{S}$ [M-H]⁻ 535.2272; found 535.2277.

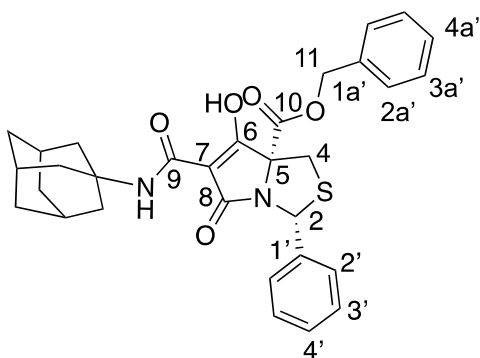
(2*R*,5*R*)-7-(Adamantylaminocarbonyl)-5-benzyloxycarbonyl-2-(5-bromopyridin-2-yl)-1-aza-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 156c



Synthesised from **105c** (1 eqv. 73 mg, 0.1 mmol) with 1-adamantylamine; yield (29 mg, 34%); pale yellow oil; $R_f = 0.78$ (EtOAc : petrol; 3 : 2); $[\alpha]_D^{25} = +86.1$ ($c = 0.97$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1743 (s, C=O), 1688 (s, C=O), 1626 (s, C=C); $^1\text{H NMR}$ (CDCl_3 , 400

MHz): a mixture of 0.9 : 1 AB/CD tautomers: δ (OH not observed), 8.35 (app dd, $J = 8.9$, 1.9 Hz, 2H, H6' AB and CD), 7.89 (br. s, 1H, NH), 7.69 (br. s, 1H, NH), 7.48 – 7.42 (m, 2H, H4' AB and CD), 7.39 – 7.29 (m, 10H, H2a', H3a' and H4a', AB and CD), 7.23 (d, $J = 7.1$ Hz, 2H, H3' AB and CD), 6.26 (s, 1H, H2 CD), 6.23 (s, 1H, H2 AB), 5.28 (app dd, $J = 12.2$, 1.6 Hz, 2H, H11_B AB and CD), 5.07 (app dd, $J = 12.2$, 7.6 Hz, 2H, H11_A AB and CD), 3.90 (app dd, $J = 11.5$, 4.0 Hz, 2H, H4_B AB and CD), 3.16 (d, $J = 11.5$ Hz, 2H, H4_A AB and CD), 2.14 (br. s, 6H, Adamantyl-CH AB and CD), 2.06 (br. s, 12H, Adamantyl-CH₂ AB and CD), 1.70 (br. s, 12H, Adamantyl-CH₂); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ 189.8 (C6 CD), 187.0 (C6 AB), 178.9 (C8 CD), 173.6 (C8 AB), 167.3 (C10 CD), 167.1 (C10 AB), 166.7 (C9), 157.4, 157.3 (C2'), 150.1 (C6'), 139.3 (C4'), 135.0, 134.9 (C1a'), 128.7, 128.7, 128.7 (C3a', C4a', C2a'), 121.6 (C3'), 120.0, 119.6 (C5'), 88.8, 84.0 (C7), 83.6, 82.0 (C5), 68.5, 68.3 (C11), 64.1, 64.0 (C2), 55.1, 54.6 (Adamantyl-C), 41.7, 41.6 (Adamantyl-CH₂), 36.0, 35.9 (Adamantyl-CH₂), 35.7, 35.6 (C4), 29.4 (Adamantyl-CH); m/z (ESI⁺) 624 and 626 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for C₃₀H₃₁O₅N₃BrS [M+H]⁺ 624.1162 and 626.1144; found 624.0368 and 626.1137.

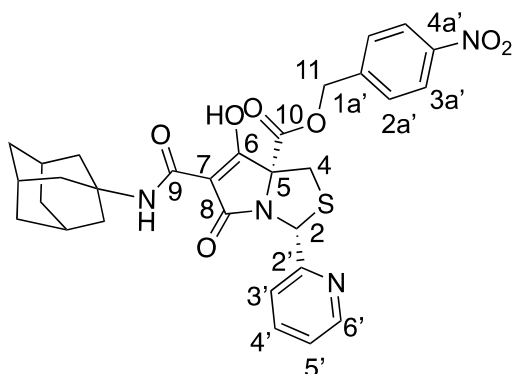
(2*R*,5*R*)-7-(Adamantylaminocarbonyl)-5-benzyloxycarbonyl-1-aza-6-hydroxy-8-oxo-2-phenyl-3-thiabicyclo[3.3.0]oct-6-ene, 156e



Synthesised from **105e** (1 eqv. 71 mg, 0.2 mmol) with 1-adamantylamine; yield (57 mg, 67%); yellow oil; $R_f = 0.78$ (EtOAc : petrol; 7 : 3); $[\alpha]_D^{25} = +65.7$ ($c = 0.95$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1742 (s, C=O), 1688 (s, C=O), 1627 (s, C=C); $^1\text{H NMR}$ (CDCl_3 , 400

MHz): a mixture of 1 : 1 AB/CD tautomers: δ (OH not observed), 7.82 (br. s, 1H, NH), 7.69 (br. s, 1H, NH), 7.48 – 7.44 (m, 4H, H2' AB and CD), 7.33 – 7.28 (m, 6H, H3' and H4', AB and CD), 7.24 – 7.17 (m, 10H, H2a', H3a' and H4a', AB and CD), 6.28 (s, 1H, H2 CD), 6.23 (s, 1H, H2 AB), 5.20 – 5.04 (m, 4H, H11 AB and CD), 3.89 (app dd, $J = 11.5, 6.4$ Hz, 2H, H4_B AB and CD), 3.11 (d, $J = 11.5$ Hz, 2H, H4_A AB and CD), 2.13 (br. s, 6H, Adamantyl-CH AB and CD), 2.05 (br. s, 12H, Adamantyl-CH₂ AB and CD), 1.69 (br. s, 12H, Adamantyl-CH₂); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ 189.3 (C6 CD), 187.0 (C6 AB), 178.9 (C8 CD), 173.6 (C8 AB), 167.5 (C10 CD), 167.2 (C10 AB), 166.7 (C9 CD), 166.6 (C9 AB), 139.1, 139.0 (C1'), 134.9, 134.8 (C1a'), 128.6, 128.6, 128.5, 128.5, 128.4, 128.4 (C3', C3a', C2a'), 128.1 (C4' and C4a'), 126.9 (C2'), 89.4, 84.1 (C7), 82.8, 81.2 (C5), 68.5, 68.3 (C11), 64.1, 63.9 (C2), 54.9, 54.4 (Adamantyl-C), 41.6, 41.6 (Adamantyl-CH₂), 36.0, 35.9 (Adamantyl-CH₂), 35.4, 35.3 (C4), 29.4 (Adamantyl-CH); m/z (ESI⁻) 543 ([M-H]⁻, 100%); HRMS (ESI⁻) m/z calcd for $\text{C}_{31}\text{H}_{31}\text{O}_5\text{N}_2\text{S}$ [M-H]⁻ 543.1959; found 543.1968.

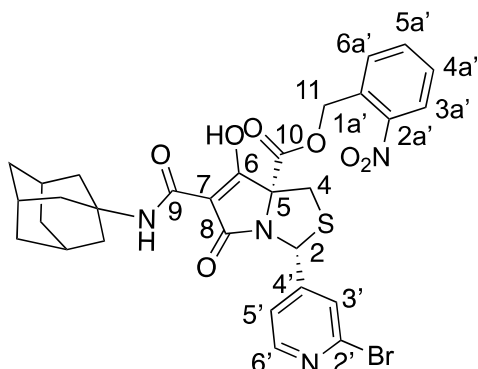
(2*R*,5*R*)-7-(Adamantylaminocarbonyl)-5-(4-nitrobenzyloxycarbonyl)-1-aza-6-hydroxy-8-oxo-2-(pyridin-2-yl)-3-thiabicyclo[3.3.0]oct-6-ene, 157a



Synthesised from **106a** (1 eqv. 42 mg, 0.08 mmol) with 1-adamantylamine; yield (26 mg, 52%); pale yellow oil; $R_f = 0.41$ (100% EtOAc); $[\alpha]_D^{25} = +142.8$ ($c = 0.87$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1745 (s, C=O), 1689 (s, C=O), 1627 (s, C=C); $^1\text{H NMR}$

(CDCl_3 , 400 MHz): a mixture of 0.8 : 1 AB/CD tautomers: δ (OH not observed), 8.40 (br. s, 2H, H6' AB and CD), 8.11 (d, $J = 8.3$ Hz, 4H, H3a' AB and CD), 7.86 (br. s, 1H, NH CD), 7.75 (br. s, 1H, NH AB), 7.58 – 7.45 (m, 4H, H4' and H3', AB and CD), 7.36 (d, $J = 8.5$ Hz, 4H, H2a' AB and CD), 7.15 – 7.05 (m, 2H, H5' AB and CD), 6.36 (s, 1H, H2 CD), 6.34 (s, 1H, H2 AB), 5.39 – 5.17 (m, 4H, H11 AB and CD), 3.94 (d, $J = 11.6$ Hz, 2H, H4_B AB and CD), 3.21 (d, $J = 11.6$ Hz, 2H, H4_A AB and CD), 2.15 (br. s, 6H, Adamantyl-CH AB and CD), 2.06 (br. s, 12H, Adamantyl-CH₂ AB and CD), 1.70 (br. s, 12H, Adamantyl-CH₂ AB and CD); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ 189.1 (C6 CD), 187.1 (C6 AB), 179.0 (C8 CD), 173.8 (C8 AB), 167.4 (C10 CD), 167.2 (C10 AB), 166.7 (C9), 158.7 (C2'), 149.1 (C6'), 147.8 (C4a'), 142.5 (C1a'), 136.9 (C4'), 128.3 (C2a'), 123.8 (C3a'), 122.8 (C3'), 120.2 (C5'), 88.4, 84.0 (C7), 83.5, 82.0 (C5), 66.5, 66.3 (C11), 64.6, 64.5 (C2), 55.2, 54.7 (Adamantyl-C), 41.7, 41.6 (Adamantyl-CH₂), 35.9 (Adamantyl-CH₂), 35.5, 35.4 (C4), 29.4 (Adamantyl-CH); m/z (ESI⁻) 589 ([M-H]⁻, 100%); HRMS (ESI⁻) m/z calcd for $\text{C}_{30}\text{H}_{29}\text{O}_7\text{N}_4\text{S}$ [M-H]⁻ 589.1762; found 589.1757.

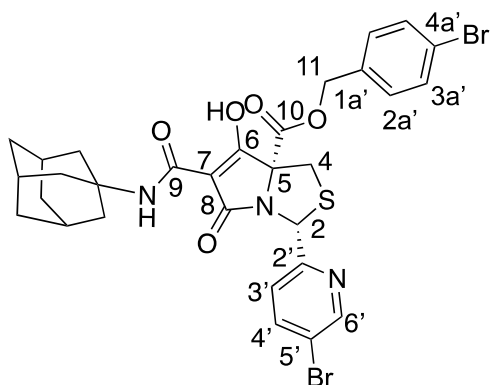
(2*R*,5*R*)-7-(Adamantylaminocarbonyl)-1-aza-2-(2-bromopyridin-4-yl)-6-hydroxy-5-(2-nitrobenzyloxycarbonyl)-8-oxo-3-thiabicyclo[3.3.0]-oct-6-ene, 158b



Synthesised from **108b** (1 eqv. 42 mg, 0.07 mmol) with 1-adamantylamine; yield (21 mg, 43%); yellow oil; $R_f = 0.20$ (100% EtOAc); $[\alpha]_D^{25} = +1.84$ ($c = 1.05$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1745 (s, C=O), 1689 (s, C=O), 1629 (s, C=C); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): a mixture of 1 :

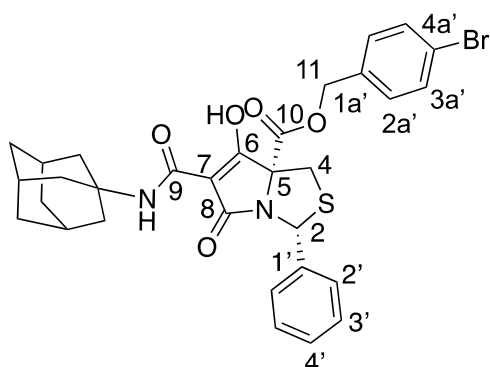
1 AB/CD tautomers: δ (NH and OH not observed), 8.32 (d, $J = 5.1$ Hz, 1H, H6'), 8.22 (d, $J = 5.1$ Hz, 1H, H6'), 8.09 (d, $J = 8.1$ Hz, 1H, H3a'), 7.89 (d, $J = 8.1$ Hz, 1H, H3a'), 7.68 (s, 1H, H3'), 7.61 (s, 1H, H3'), 7.55 (d, $J = 7.7$ Hz, 2H, H5a' AB and CD), 7.48 (t, $J = 7.7$ Hz, 2H, H4a' AB and CD), 7.41 (d, $J = 7.7$ Hz, 2H, H6a' AB and CD), 7.38 (d, $J = 5.1$ Hz, 1H, H5'), 7.34 (d, $J = 5.1$ Hz, 1H, H5'), 6.18 (s, 1H, H2 CD), 6.14 (s, 1H, H2 AB), 5.63 – 5.56 (m, 4H, H11 AB and CD), 3.89 – 3.84 (m, 2H, H4_B AB and CD), 3.14 (d, $J = 11.6$ Hz, 2H, H4_A AB and CD), 2.15 (br. s, 6H, Adamantyl-CH AB and CD), 2.06 (br. s, 12H, Adamantyl-CH₂ AB and CD), 1.71 (br. s, 12H, Adamantyl-CH₂ AB and CD); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ 189.7 (C6 CD), 186.5 (C6 AB), 178.5 (C8 CD), 173.5 (C8 AB), 167.0 (C10 CD), 166.8 (C10 AB), 166.7 (C9 CD), 166.7 (C9 AB), 151.5 (C6'), 150.3, 150.3 (C4'), 147.5 (C2a'), 142.6 (C2'), 134.0 (C6a'), 132.6 (C1a'), 129.3, 129.3 (C5a'), 129.1, 129.0 (C4a'), 125.9, 125.9 (C3'), 125.3, 125.3 (C3a'), 121.0, 120.9 (C5'), 87.6, 82.7 (C7), 81.5, 81.1 (C5), 65.4, 65.2 (C11), 63.4, 63.2 (C2), 55.4, 55.1 (Adamantyl-C), 41.6 (Adamantyl-CH₂), 36.0, 35.9 (Adamantyl-CH₂), 35.7, 35.7 (C4), 29.4 (Adamantyl-CH); m/z (ESI⁻) 667 and 669 ([M-H]⁻, 100%); HRMS (ESI⁻) m/z calcd for $\text{C}_{30}\text{H}_{28}\text{O}_7\text{N}_4\text{BrS}$ [M-H]⁻ 667.0868 and 669.0847; found 667.0860 and 669.0840.

(2*R*,5*R*)-7-(Adamantylaminocarbonyl)-1-aza-5-(4-bromobenzoyloxycarbonyl)-2-(5-bromopyridin-2-yl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 159c



Synthesised from **109c** (1 eqv. 40 mg, 0.06 mmol) with 1-adamantylamine; yield (20 mg, 44%); pale yellow oil; $R_f = 0.54$ (100% EtOAc); $[\alpha]_D^{25} = +144$ ($c = 1.0$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1743 (s, C=O), 1689 (s, C=O), 1627 (s, C=C); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): a mixture of 0.9 : 1 AB/CD tautomers: δ 8.36 (app dd, $J = 8.0, 2.0$ Hz, 2H, H6' AB and CD), 7.87 (br. s, 1H, NH), 7.70 (br. s, 1H, NH), 7.56 (app dt, $J = 8.4, 2.4$ Hz, 2H, H4' AB and CD), 7.44 (d, $J = 8.3$ Hz, 4H, H3a' AB and CD), 7.35 (d, $J = 8.4$ Hz, 2H, H3' AB and CD), 7.11 (d, $J = 8.3$ Hz, 4H, H2a' AB and CD), 6.27 (s, 1H, H2 CD), 6.23 (s, 1H, H2 AB), 5.63 (br. s, 2H, OH AB and CD), 5.21 (app dd, $J = 12.4, 2.6$ Hz, 2H, H11_B AB and CD), 5.03 (app dd, $J = 12.4, 6.2$ Hz, 2H, H11_A AB and CD), 3.89 (app dd, $J = 11.6, 4.1$ Hz, 2H, H4_B AB and CD), 3.17 (app dd, $J = 11.6, 1.1$ Hz, 2H, H4_A AB and CD), 2.15 (br. s, 6H, Adamantyl-CH AB and CD), 2.06 (br. s, 12H, Adamantyl-CH₂ AB and CD), 1.70 (br. s, 12H, Adamantyl-CH₂); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ 189.9 (C6 CD), 187.0 (C6 AB), 178.9 (C8 CD), 173.6 (C8 AB), 167.3 (C10 CD), 167.1 (C10 AB), 166.7 (C9 CD), 166.7 (C9 AB), 157.5, 157.3 (C2'), 150.2 (C6'), 139.3 (C4'), 134.1, 133.9 (C1a'), 131.9 131.8 (C3a'), 130.2 (C2a'), 122.8, 122.7 (C4a'), 121.6 (C3'), 119.8, 119.8 (C5'), 88.5, 83.9 (C7), 83.5, 82.0 (C5), 67.6, 67.4 (C11), 64.1, 64.0 (C2), 55.2, 54.7 (Adamantyl-C), 41.7, 41.6 (Adamantyl-CH₂), 36.0, 35.9 (Adamantyl-CH₂), 35.6, 35.5 (C4), 29.4 (Adamantyl-CH); m/z (ESI⁺) 702 (50%), 704 ([M+H]⁺, 100%) and 706 (50%); HRMS (ESI⁺) m/z calcd for $\text{C}_{30}\text{H}_{30}\text{O}_5\text{N}_3\text{Br}_2\text{S}$ [M+H]⁺ 702.0267, 704.0249 and 706.0232; found 702.0265, 704.0243 and 706.0220.

(2*R*,5*R*)-7-(Adamantylaminocarbonyl)-1-aza-5-(4-bromobenzoyloxycarbonyl)-6-hydroxy-8-oxo-2-phenyl-3-thiabicyclo[3.3.0]oct-6-ene, 159e

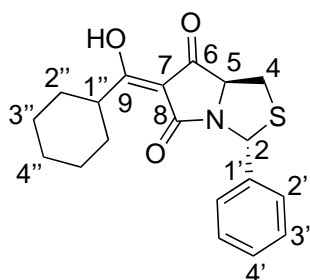


Synthesised from **109e** (1 eqv. 29 mg, 0.05 mmol) with 1-adamantylamine; yield (16 mg, 47%); pale yellow oil; $R_f = 0.29$ (EtOAc : petrol; 2 : 3); $[\alpha]_D^{25} = +152$ ($c = 0.80$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1745 (s, C=O), 1689 (s, C=O), 1626 (s, C=C); $^1\text{H NMR}$ (CDCl_3 , 400

MHz): a mixture of 0.9 : 1 AB/CD tautomers: δ 7.80 (br. s, 1H, NH), 7.71 (br. s, 1H, NH), 7.46 – 7.39 (m, 8H, H2' and H3a', AB and CD), 7.25 – 7.20 (m, 6H, H3' and H4', AB and CD), 7.04 (app t, $J = 7.8$ Hz, 4H, H2a' AB and CD), 6.29 (s, 1H, H2 CD), 6.24 (s, 1H, H2 AB), 5.79 (br. s, 2H, OH AB and CD), 5.11 – 5.01 (m, 4H, H11 AB and CD), 3.87 (app dd, $J = 11.5, 6.7$ Hz, 2H, H4_B AB and CD), 3.11 (d, $J = 11.5$ Hz, 2H, H4_A AB and CD), 2.14 (br. s, 6H, Adamantyl-CH AB and CD), 2.05 (br. s, 12H, Adamantyl-CH₂ AB and CD), 1.70 (br. s, 12H, Adamantyl-CH₂); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ 189.4 (C6 CD), 186.9 (C6 AB), 178.8 (C8 CD), 173.6 (C8 AB), 167.4 (C10 CD), 167.2 (C10 AB), 166.8 (C9 CD), 166.7 (C9 AB), 139.1, 139.0 (C1'), 134.0, 133.9 (C1a'), 131.8, 131.8 (C3a'), 130.1, 130.1 (C2a'), 128.5, 128.5 (C3'), 128.2 (C4'), 127.0, 127.0 (C2'), 122.7, 122.6 (C4a'), 88.8, 84.0 (C7), 82.8, 81.2 (C5), 67.6, 67.4 (C11), 64.0, 63.9 (C2), 55.1, 54.6 (Adamantyl-C), 41.7, 41.7 (Adamantyl-CH₂), 36.0, 36.0 (Adamantyl-CH₂), 35.4, 35.3 (C4), 29.4 (Adamantyl-CH); m/z (ESI⁻) 621 and 623 ([M-H]⁻, 100%); HRMS (ESI⁻) m/z calcd for C₃₁H₃₀O₅N₂BrS [M-H]⁻ 621.1064 and 623.1046; found 621.1073 and 623.1052.

Tetramate analogues **160-162** were synthesised from bromo-substituted tetramate ketones **129e**, **142e** and tetramate carboxamide **159c** respectively according the modified Suzuki-Miyaura cross-coupling conditions (general procedure **Q**, 6.4.6).

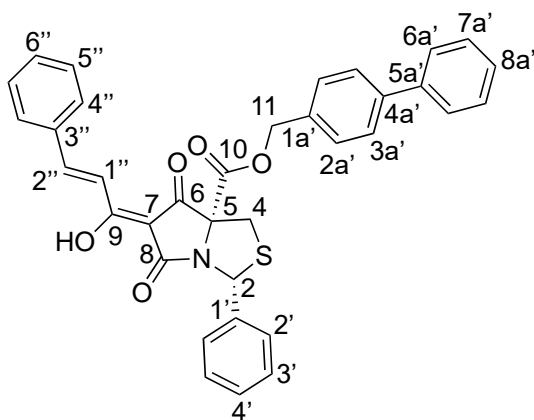
(2*R*,5*S*)-1-Aza-7-[(*E*)-cyclohexyl(hydroxy)methylene]-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]-octane, 160e



Synthesised by general procedure **Q** with **129e** (1 eqv., 55 mg, 0.01 mmol), aq. Na₂CO₃ (2M, 6.3 eqv.), Pd(OAc)₂ (0.05 eqv.) and Xphos (0.15 eqv.); yield (14 mg, 41%); brown oil; *R_f* = 0.18 (EtOAc : petrol; 7 : 3); [α]_D²⁵ = +146 (*c* = 0.40, CHCl₃); *v*_{max}/cm⁻¹

1710 (s, C=O), 1649 (s, C=O), 1605 (s, C=C); ¹H NMR (CDCl₃, 400 MHz): a mixture of 2.4 : 1 AB/CD tautomer: δ (*OH* not observed), 7.46 (d, *J* = 6.8 Hz, 2H, H2'), 7.39 – 7.29 (m, 3H, H4' and H3'), 6.45 (s, H2 minor, CD), 6.42 (s, 1H, H2 major, AB), 4.58 (dd, *J* = 9.1, 7.0 Hz, H5 minor, CD), 4.46 (dd, *J* = 9.5, 6.9 Hz, 1H, H5 major, AB), 3.43 – 3.30 (m, 2H, H4_B and H1''), 3.01 (dd, *J* = 10.9, 9.5 Hz, 1H, H4_A), 1.88 – 1.69 (m, 6H, H3'' and H2''), 1.60 – 1.47 (m, 2H, H3'), 1.45 – 1.30 (m, 2H, H4''); ¹³C{¹H} NMR (CDCl₃, 126 MHz): δ 196.5 (C6 AB), 193.7 (C6 CD), 192.5 (C9 AB), 191.4 (C9 CD), 175.5 (C8 AB), 168.4 (C8 CD), 140.4 (C1' CD), 139.9 (C1' AB), 129.9, 129.1, 128.9, 128.8, 128.5, 128.3 (C3' and C4'), 126.7 (C2' CD), 126.5 (C2' AB), 103.3 (C7 CD), 100.5 (C7 AB), 70.5 (C5 AB), 67.8 (C5 CD), 62.8 (C2 CD), 62.3 (C2 AB), 41.1 (C1''), 33.6 (C4 AB), 33.5 (C4 CD), 28.9, 28.7 (C2''), 25.7, 25.6, 25.5 (C3'' and C4'); *m/z* (ESI⁺) 344 ([M+H]⁺, 100%); HRMS (ESI⁺) *m/z* calcd for C₁₉H₂₂O₃NS [M+H]⁺ 344.1315; found 344.1314.

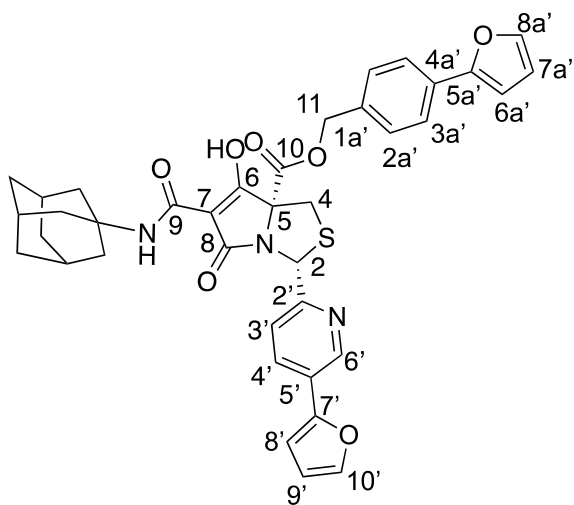
(2*R*,5*R*)-1-Aza-5-(1,1'-biphenyloxycarbonyl)-7-[(*Z*,2*E*)-1-hydroxy-3-phenylallylidene]-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]-octane, 161e



Synthesised by general procedure **Q** with **142e** (1 eqv., 16 mg, 0.03 mmol), Pd(OAc)₂ (0.05 eq.) and Xphos (0.15 eq.); Yield (8 mg, 50%); orange oil; *R*_f = 0.42 (petrol: EtOAc; 3 : 7); [α]_D²⁵ = +73.0 (*c* = 0.80, CHCl₃); *v*_{max}/cm⁻¹ 1748 (s, C=O), 1707 (s, C=O), 1652 (s, C=O), 1624 (s,

C=C), 1580 (s, C=C), 1562 (s, C=C); ¹H NMR (CDCl₃, 400 MHz): a 0.32 : 1 mixture of AB/CD tautomers: δ (*OH* not observed), 7.96 (d, *J* = 16.0 Hz, 1H, H1''), 7.78 – 7.74 (m, 2H, H6a'), 7.71 (d, *J* = 16.0 Hz, 1H, H2''), 7.68 – 7.64 (m, 2H, H4''), 7.53 – 7.37 (m, 8H, H2', H3', H5'' and H7a'), 7.29 – 7.21 (m, 5H, H4', H3a', H8a' and H6''), 6.99 (d, *J* = 8.5 Hz, 2H, H2a'), 6.49 (s, H2 minor tautomer), 6.44 (s, 1H, H2 major tautomer), 5.01 (s, 2H, H11), 3.98 (d, *J* = 11.4 Hz, 1H, H4_B), 3.14 (d, *J* = 11.4 Hz, 1H, H4_A); ¹³C{¹H} NMR (CDCl₃, 126 MHz): δ 196.1 (C6 AB), 187.3 (C6 CD), 176.8 (C9 AB), 176.4 (C9 CD), 176.2 (C8 CD), 166.7 (C10), 147.1 (C2''), 138.2 (C1'), 135.8 (C5a'), 134.2 (C3''), 133.7 (C1a'), 132.9 (C4a'), 132.0 (C3a'), 131.9 (C4''), 131.3 (C7a'), 130.1 (C2a'), 129.5, 129.3 (C3' and C5''), 128.7, 128.6, 128.1 (C4', C6'', C6a'), 127.2 (C2'), 122.8 (C8a'), 117.3 (C1''), 99.4 (C7), 82.4 (C5), 67.8 (C11), 64.0 (C2), 35.6 (C4); *m/z* (ESI⁻) 572 ([M-H]⁻, C₃₅H₂₆O₅NS, 100%).

(2*R*,5*R*)-7-(Adamantylaminocarbonyl)-1-aza-5-[4-(furan-2-yl)benzyloxycarbonyl]-2-[5-(furan-2-yl)pyridin-2-yl]-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 162c



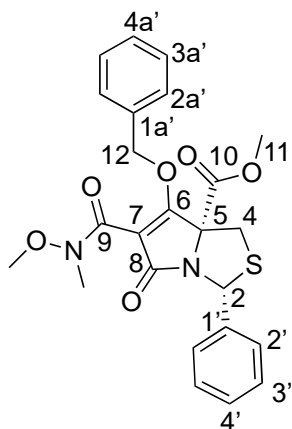
Synthesised by general procedure **Q** with **159c** (1 eqv., 10 mg, 0.01 mmol), RuPhos-Pd-G2 (0.15 eqv.); yield (6 mg, 62%); yellow oil; $R_f = 0.25$ (EtOAc : petrol; 1 : 1); $[\alpha]_D^{25} = +121$ ($c = 0.60$, CHCl_3); $\nu_{\text{max}}/\text{cm}^{-1}$ 1743 (s, C=O), 1687 (s, C=O), 1628 (s, C=C); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): a mixture of 1 : 1 AB/CD

tautomers: δ (OH not observed), 8.65 (br. s, 1H, NH), 7.93 – 7.84 (m, 2H, H6' AB and CD), 7.73 (br. s, 1H, NH), 7.67 (d, $J = 8.1$ Hz, 2H, H4' AB and CD), 7.57 (d, $J = 8.4$ Hz, 4H, H3a' AB and CD), 7.55 – 7.36 (m, 6H, H3', H8a' and H10', AB and CD), 7.11 (d, $J = 8.4$ Hz, 4H, H2a' AB and CD), 6.69 (d, $J = 4.2$ Hz, 2H, H8' AB and CD), 6.59 (d, $J = 8.3$ Hz, 2H, H6a' AB and CD), 6.53 – 6.45 (m, 4H, H7a' and H9', AB and CD), 6.35 (s, 1H, H2 CD), 6.32 (s, 1H, H2 AB), 5.21 (d, $J = 12.3$ Hz, 2H, H11_B AB and CD), 5.05 (app dd, $J = 12.3, 5.2$ Hz, 2H, H11_A AB and CD), 3.93 (app dd, $J = 11.5, 3.9$ Hz, 2H, H4_B AB and CD), 3.20 (d, $J = 11.6$, 2H, H4_A AB and CD), 2.15 (br. s, 6H, Adamantyl-CH AB and CD), 2.07 (br. s, 12H, Adamantyl-CH₂ AB and CD), 1.71 (br. s, 12H, Adamantyl-CH₂ AB and CD); $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 151 MHz): δ 190.0 (C6 CD), 187.3 (C6 AB), 179.1 (C8 CD), 173.8 (C8 AB), 167.5 (C10 CD), 167.2 (C10 AB), 166.8 (C9 CD), 166.7 (C9 AB), 157.2, 157.1, 157.0 (C2', C5a' and C7'), 150.9, 150.2 (C6'), 144.8, 143.2 (C8a' and C10'), 139.3 (C4'), 134.2 (C1a'), 131.8 (C5'), 131.5 (C4a'), 130.2 (C2a'), 123.9, 123.9 (C3a'), 122.6 (C3'), 111.9, 111.9 (C7a' and C9'), 107.6, 106.8 (C6a' and C8'), 88.9, 84.1 (C7), 83.5, 81.9 (C5), 67.5, 67.3 (C11), 64.6, 64.4 (C2), 55.1, 54.6

(Adamantyl-C), 41.7, 41.7 (Adamantyl-CH₂), 36.0, 36.0 (Adamantyl-CH₂), 35.5, 35.4 (C4), 29.5 (Adamantyl-CH); *m/z* (ESI⁺) 716 ([M+K]⁺, 100%, C₃₈H₃₅O₇N₃SK⁺).

(2*R*,5*R*)-1-Aza-6-benzyloxy-5-methoxycarbonyl-7-(methoxy(methyl)carbamoyl)-8-oxo-2-phenyl-3-thiabicyclo[3.3.0]-oct-6-ene, 163e

Synthesised from **101e** (1 eqv., 150 mg, 0.4 mmol) by stirring in a mixture of benzyl bromide (1.2 eqv.), powdered K₂CO₃ (5 eqv.) and catalytic benzyltributylammonium chloride (0.2 eqv.) in CHCl₃ (*c* = 0.15) at room temperature for 18 h. The reaction mixture was washed sequentially with 2M HCl solution, sat. aq. NaHCO₃ and brine. The combined organic layer was dried with MgSO₄, filtered and concentrated *in vacuo*. The resulting residue was purified with column chromatography to produce **163e**.

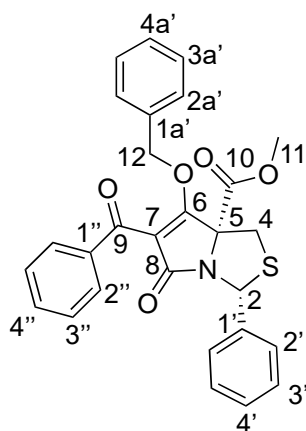


Yield (44 mg, 24%); pale yellow oil; *R_f* = 0.34 (EtOAc : petrol; 1 : 1);

¹H NMR (CDCl₃, 400 MHz): δ 7.49 – 7.27 (m, 10H, H_{2a'}, H_{3a'}, H_{4a'}, H_{2'}, H_{3'} and H_{4'}), 6.26 (s, 1H, H₂), 5.34 – 5.21 (m, 2H, H₁₂), 3.86 (d, *J* = 11.5 Hz, 1H, H_{4B}), 3.68 (s, 3H, NOCH₃), 3.46 (s, 3H, H₁₁), 3.26 (s, 3H, NCH₃), 3.16 (d, *J* = 11.5 Hz, 1H, H_{4A}); ¹³C NMR (CDCl₃, 101 MHz): δ (C₆ and C₈ not shown), 168.1 (C₁₀), 162.7 (C₉), 139.

1 (C_{1'}), 134.6 (C_{1a'}), 129.1, 128.8, 128.5, 128.2, 127.9 (C_{2a'}, C_{3a'}, C_{4a'}, C_{3'} and C_{4'}), 126.9 (C_{2'}), 78.9 (C₇), 74.5 (C₁₂), 63.5, 63.5 (C₅ and C₂), 61.5 (NOCH₃), 53.4 (C₁₁), 35.0 (C₄), 32.4 (NCH₃); *m/z* (ESI⁺) 469 ([M+H]⁺, 100%); HRMS (ESI⁺) *m/z* calcd for C₂₄H₂₅O₆N₂S [M+H]⁺ 469.1428; found 469.1429.

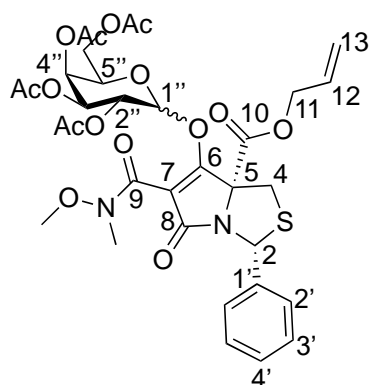
(2*R*,5*R*)-1-Aza-7-benzoyl-6-benzyloxy-5-methoxycarbonyl-8-oxo-2-phenyl-3-thiabicyclo[3.3.0]-oct-6-ene, 164e



Synthesised from **163e** (40 mg, 0.08 mmol) by general procedure **P** with phenylmagnesium bromide; yield (10 mg, 25%); yellow oil; $R_f = 0.50$ (EtOAc : petrol; 1 : 4); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ 7.77 (dd, $J = 8.3, 1.2$ Hz, 2H), 7.63 – 7.26 (m, 7H), 7.23 – 7.16 (m, 4H), 7.09 (dd, $J = 7.6, 2.0$ Hz, 2H), (H2', H3', H4', H2'', H3'', H4'', H2a', H3a' and H4a', a total of 15H), 6.23 (s, 1H,

H2), 5.32 (d, $J = 12.0$ Hz, 1H, H12_B), 5.02 (d, $J = 12.0$ Hz, 1H, H12_A), 3.93 (d, $J = 11.5$ Hz, 1H, H4_B), 3.76 (s, 3H, H11), 3.23 (d, $J = 11.5$ Hz, 1H, H4_A); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz): δ 190.4 (C9), 181.3 (C6), (C8 not shown), 163.0 (C10), 138.9 (C1'), 137.0 (C1''), 134.0 (C1a'), 129.9, 129.1, 128.9, 128.7, 128.6, 128.6, 128.4, 128.0 (C2a', C3a', C4a', C3', C4', C3'' and C4''), 127.3, 127.2 (C2' and C2''), (C7 not shown), 79.0 (C5), 76.1 (C12), 63.8 (C2), 53.7 (C11), 35.3 (C4); m/z (ESI⁺) 486 ([M+H]⁺, 100%); HRMS (ESI⁺) m/z calcd for $\text{C}_{28}\text{H}_{24}\text{O}_5\text{NS}$ [M+H]⁺ 486.1370; found 486.1367.

(2*R*,5*R*)-5-Allyloxycarbonyl-1-aza-6-(2,3,4,6-*tetra-O*-acetyl- β -D-galactopyranosyl)-7-(methoxy(methyl)carbamoyl)-8-oxo-2-phenyl-3-thiabicyclo[3.3.0]-oct-6-ene, 165



Synthesised from **102e** (156 mg, 0.4 mmol) according to a literature published protocol with TMSOTf and 2,3,4,6-*tetra-O*-acetyl- β -D-galactopyranosyl trichloacetimidate;¹⁹⁶ yield (164 mg, 58%); yellow oil; $R_f = 0.34$ (EtOAc : petrol; 1 : 1); $^1\text{H NMR}$ (CDCl_3 , 400 MHz): a 1.3 : 1 mixture of isomers:

δ 7.47 (d, $J = 7.4$ Hz, 2H, H2'), 7.36 – 7.27 (m, 3H, H3' and H4'), 6.25 (s, 1H, H2), 6.12 (d, $J =$

3.5 Hz, H1'' minor), 5.97 (d, $J = 3.2$ Hz, 1H, H1'' major), 5.94 – 5.82 (m, H12 minor), 5.81 – 5.67 (m, 1H, H12 major), 5.53 – 5.14 (m, 6H, H5, H13, H2'', H3'' and H4''), 4.69 – 4.55 (m, 2H, H11), 4.27 – 4.06 (m, 3H, H5'' and H6''), 3.94 (d, $J = 11.3$ Hz, 1H, H4_B major), 3.87 (d, $J = 11.5$ Hz, H4_B minor), 3.74 (s, NOCH₃ minor), 3.71 (s, 3H, NOCH₃ major), 3.25 (s, 3H, NCH₃ major), 3.23 (s, NCH₃ minor), 3.22 – 3.12 (m, 1H, H4_A), 2.14 (s, 3H, OAc), 2.09 (s, 3H, OAc), 2.05 (s, 3H, OAc), 1.99 (s, 3H, OAc); ¹³C NMR (CDCl₃, 101 MHz): δ 70.9, 170.8, 170.3, 170.2, 170.0, 169.8, 169.6 (OAc x 4, C6 and C8), 167.1, 166.6 (C10), 163.9, 163.8 (C9), 138.7, 138.5 (C1'), 130.7, 130.7 (C12), 128.4, 128.4 (C3'), 128.2, 128.1 (C4'), 126.8, 126.6 (C2'), 120.3, 119.5 (C13), 97.8, 96.6 (C1''), 78.7, 78.7 (C7), 72.5 (C5''), 70.8, 70.6 (C3''), 68.3, 67.6, 67.2, 66.9, 66.7, 65.9 (C2'', C4'', C5, C2 and C11), 63.3, 63.1, 61.9, 61.7 (NOCH₃), 59.9, 59.6 (C6''), 35.3, 34.8 (C4), 32.4, 32.3 (NCH₃), 20.6, 20.6, 20.5 (OAc x 4); m/z (ESI⁺) 757 ([M+Na]⁺, 100%); HRMS (ESI⁺) m/z calcd for C₃₃H₃₈O₁₅N₂NaS [M+Na]⁺ 757.1885; found 757.1881.

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Appendix: Physiochemical properties and activity of synthesised tetramate analogues.^a

Cpd No.	MW /Da	clogP	clogD _{7.4}	PSA /Å ²	MSA /Å ²	% PSA	HBD	HBA	MIC against MRSA	
									µg/ml	µM
GM 18	400.88	3.21	-0.40	69.64	482.40	14.4	2	3	3.91	9.75
GM 22	410.53	1.78	-0.47	69.64	542.94	12.8	2	3	0.98	2.39
25a	306.34	0.72	-0.06	79.73	385.50	20.7	1	4	-	-
25b	385.23	1.64	0.47	79.73	403.83	19.7	1	4	-	-
25c	385.23	1.51	0.37	79.73	403.83	19.7	1	4	-	-
25d	340.78	1.34	0.21	79.73	399.94	19.9	1	4	-	-
27a	401.87	2.17	-1.46	82.53	475.00	17.4	2	4	-	-
27b	480.76	3.09	-0.57	82.53	495.37	16.7	2	4	-	-
27c	480.76	2.96	-0.69	82.53	495.65	16.7	2	4	-	-
27d	436.31	2.79	-0.87	82.53	491.50	16.8	2	4	-	-
28a	359.44	0.61	-1.87	82.53	475.29	17.4	2	4	-	-
28b	438.34	1.53	-1.32	82.53	495.95	16.6	2	4	-	-
28c	438.34	1.41	-1.42	82.53	496.41	16.6	2	4	7.81	17.8
28d	393.89	1.23	-1.58	82.53	491.87	16.8	2	4	-	-
29b	440.31	-0.22	-3.15	91.76	481.53	19.1	2	5	-	-
29c	440.31	-0.35	-3.26	91.76	481.70	19.0	2	5	-	-
30a	411.52	0.75	-1.71	82.53	535.87	15.4	2	4	1.95	4.74
30b	490.42	1.66	-1.12	82.53	555.65	14.9	2	4	1.95	3.98
30c	490.42	1.54	-1.23	82.53	556.52	14.8	2	4	3.91	7.97
30d	445.96	1.36	-1.38	82.53	552.09	14.9	2	4	0.24	0.538
32	309.38	2.82	2.82	50.27	397.45	12.6	0	3	-	-
35	311.36	1.14	-2.38	66.32	375.60	17.7	1	4	-	-
36e	414.90	3.14	3.14	58.64	522.40	11.2	1	3	-	-
37a	425.55	2.55	2.55	71.53	576.01	12.4	1	5	-	-
37c	504.44	3.35	3.35	71.53	596.60	12.0	1	5	-	-
37e	424.56	3.59	3.59	58.64	583.38	10.1	1	4	-	-
38a	439.57	2.90	2.90	71.53	606.18	11.8	1	5	-	-
38c	518.47	3.69	3.69	71.53	627.25	11.4	1	5	-	-
38e	438.59	3.93	3.93	58.64	614.18	9.54	1	4	-	-
39a-1	453.60	3.31	3.31	71.53	637.88	11.2	1	5	-	-
39a-2	453.60	1.44	1.44	71.53	638.11	11.2	1	4	-	-
39c-1	532.50	4.10	4.10	71.53	658.51	10.9	1	5	-	-
39c-2	532.50	2.23	2.23	71.53	659.15	10.9	1	4	-	-
39e-1	452.61	4.35	4.35	58.64	645.93	9.08	1	4	-	-
39e-2	452.61	2.47	2.47	58.64	645.69	9.08	1	3	-	-
40a-1	451.59	3.29	3.29	71.53	606.47	11.8	1	5	31.3	69.2
40a-2	451.59	1.42	1.42	71.53	603.19	11.9	1	4	-	-
40c	530.48	4.09	4.09	71.53	606.47	11.8	1	5	-	-

Cpd No.	MW /Da	clogP	clogD _{7.4}	PSA /Å ²	MSA /Å ²	% PSA	HBD	HBA	MIC against MRSA	
									µg/ml	µM
40e-1	450.60	4.33	4.33	58.64	613.95	9.55	1	4	-	-
40e-2	450.60	2.46	2.46	58.64	611.04	9.60	1	3	-	-
40e'	450.60	4.21	4.21	66.48	624.09	10.7	1	3	-	-
41b	558.54	4.85	4.85	79.37	699.89	11.3	1	4	15.6	27.9
41e, 7R/S 1:2	478.65	4.98	4.98	66.48	687.00	9.68	1	3	1.95	4.07
41e, 7R/S 1:1.5	478.65	4.98	4.98	66.48	687.00	9.68	1	3	3.91	8.17
41e, 7S	478.65	4.98	4.98	66.48	687.00	9.68	1	3	7.81	16.3
48c	480.38	1.19	1.19	80.76	550.46	14.7	1	5	-	-
49c	522.46	2.03	2.03	80.76	642.00	12.6	1	5	-	-
50c	588.56	2.84	2.84	80.76	734.57	11.0	1	5	-	-
54e	487.01	2.16	2.16	75.71	626.09	12.1	1	3	-	-
55e	482.60	4.41	4.41	84.94	658.28	12.9	1	5	31.3	64.7
56e	544.67	5.84	5.84	84.94	733.51	11.6	1	5	15.6	28.6
111b	577.49	5.98	3.04	87.57	687.56	12.7	1	5	-	-
112e	347.39	1.74	-0.84	83.91	449.36	18.7	1	4	-	-
113e	373.42	2.48	-0.04	83.91	482.08	17.4	1	4	-	-
114e	375.44	2.49	0.00	83.91	510.79	16.4	1	4	-	-
115e	415.50	3.32	0.81	83.91	572.38	14.7	1	4	62.5	150
116e	423.48	3.51	0.92	83.91	557.01	15.1	1	4	-	-
117e	502.38	4.31	1.66	83.91	578.02	14.5	1	4	-	-
118e	395.43	2.70	1.38	83.91	493.11	17.0	1	4	-	-
119e	421.47	3.44	2.15	83.91	525.97	16.0	1	4	-	-
120e	423.48	3.46	2.18	83.91	554.49	15.1	1	4	-	-
121e	463.55	4.29	3.00	83.91	616.32	13.6	1	4	31.3	67.4
122e	471.53	4.48	3.16	83.91	601.00	14.0	1	4	-	-
123e	401.48	2.82	0.09	83.91	539.52	15.6	1	4	15.6	38.9
124b	495.39	3.04	-0.10	96.80	586.45	16.5	1	5	-	-
125e	427.52	3.56	0.88	83.91	572.86	14.6	1	4	7.81	18.3
126e	429.53	3.58	0.91	83.91	602.20	13.9	1	4	7.81	18.2
127e	469.50	4.41	1.72	83.91	662.98	12.7	1	4	3.91	8.33
128c	557.46	4.36	1.21	96.80	660.57	14.7	1	5	15.6	28.0
128e	477.58	4.60	1.84	83.91	647.55	13.0	1	4	3.91	8.19
129e	556.47	5.39	2.60	83.91	668.03	12.6	1	4	1.95	3.50
130e	423.48	3.35	0.76	83.91	554.67	15.1	1	4	15.6	36.8
131e	449.52	4.09	1.54	83.91	587.27	14.2	1	4	0.98	2.18
132e	451.54	4.11	1.58	83.91	616.53	13.6	1	4	3.91	8.66
133e	491.60	4.94	2.39	83.91	677.79	12.4	1	4	0.98	1.99
134e	499.59	5.13	2.51	83.91	662.21	12.7	1	4	3.91	7.83

Cpd No.	MW /Da	clogP	clogD _{7.4}	PSA /Å ²	MSA /Å ²	% PSA	HBD	HBA	MIC against MRSA	
									µg/ml	µM
135e	578.48	5.92	3.27	83.91	682.87	12.3	1	4	15.6	27.2
136e	421.47	3.48	1.20	83.91	526.62	15.9	1	4	15.6	37.1
137b	515.38	3.70	0.96	96.80	572.87	16.9	1	5	-	-
138e	447.51	4.22	1.98	83.91	559.30	15.0	1	4	7.81	17.5
139e	449.52	4.24	2.02	83.91	588.30	14.3	1	4	1.95	4.33
140e	489.59	5.07	2.83	83.91	650.24	12.9	1	4	1.95	3.98
141c	577.45	5.01	2.28	96.80	647.81	14.9	1	5	15.6	27.1
141e	497.57	5.26	2.95	83.91	634.90	13.2	1	4	3.91	7.86
142e	576.46	6.05	3.70	83.91	655.43	12.8	1	4	0.98	1.70
143e	422.46	2.13	0.80	96.80	519.29	18.6	1	5	-	-
144e	344.40	1.88	0.31	70.50	387.63	18.2	1	4	-	-
145e	416.49	1.21	-2.33	95.94	560.12	17.1	2	4	62.5	150
146e	442.53	1.95	-1.58	95.94	593.37	16.2	2	4	15.6	35.3
147e	444.55	1.96	-1.56	95.94	621.65	15.4	2	4	15.6	35.1
148e	484.61	2.79	-0.72	95.94	684.24	14.0	2	4	3.91	8.05
149c	572.47	2.74	-0.88	108.83	679.81	16.0	2	5	-	-
149e	492.59	2.99	-0.54	95.94	668.10	14.4	2	4	15.6	31.7
150e	571.49	3.78	0.18	95.94	687.88	13.9	2	4	62.5	109
151e	468.57	1.34	-2.20	95.94	620.19	15.5	2	4	15.6	33.3
152b	562.48	1.56	-2.06	108.83	666.16	16.3	2	5	31.3	55.6
153e	494.61	2.08	-1.44	95.94	655.45	14.6	2	4	15.6	31.6
154e	496.62	2.10	-1.42	95.94	680.97	14.1	2	4	7.81	15.7
155a	537.68	1.89	-1.66	108.83	736.12	14.8	2	5	7.81	14.5
155e	536.69	2.93	-0.59	95.94	745.30	12.9	2	4	62.5	116
156c	624.55	2.87	-0.74	108.83	741.90	14.7	2	5	-	-
156e	544.67	3.12	-0.41	95.94	731.18	13.1	2	4	62.5	115
157a	590.65	2.04	-1.58	151.97	764.00	19.9	2	7	31.3	52.9
158b	669.55	2.95	-0.69	151.97	781.59	19.4	2	7	62.5	93.3
159c	703.45	3.67	0.03	108.83	762.27	14.3	2	5	-	-
159e	623.56	3.91	0.32	95.94	751.84	12.8	2	4	-	-
160e	343.44	3.14	0.60	57.61	464.12	12.4	1	3	15.6	45.5
161e	573.66	6.94	4.61	83.91	745.11	11.3	1	4	3.91	6.82
162	677.77	3.35	-0.23	135.11	883.25	15.3	2	5	-	-
164e	485.55	3.47	3.47	72.91	638.28	11.4	0	4	-	-

[a] MW was calculated by ChemDraw 19.1.1.32; clogP, clogD_{7.4}, PSA, MSA, hydrogen bond donors (HBD) and acceptors (HBA) were calculated with Marvin (20.3.0), 2020, ChemAxon; % PSA was calculated by % PSA/MSA.